Hemodynamic effects of antihypertensive drugs in conscious spontaneously hypertensive rats

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Hubert Nicolas Maria Willem Nievelstein
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Promotor: Prof. dr. H.A.J. Struyker Boudier

Co-promotor: Dr. J.F.M. Smits

Referent: Prof. dr. K.H. Rahn
Referent: Prof. dr. P.A. van Zwieten

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Experimental assistance: C. Tyssen, M. Schaefer, J. Debets, H. van Essen and R. Hornsveld.

The manuscript was typed by E. Geurts and M. Hogenboom.

Graphic advises: M. Uitendaal.
Druk: Groenevelt bv, Landgraaf

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Omnes enim causae effectuum naturalium dantur per lineas,
angulos et figuras. Aliter enim impossibile est scire propter quid in illis.

Uit: Il Nome della Rosa, Umberto Eco

Ter nagedachtenis aan:
Christian Johann Doppler (1803-1853)

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ABBREVIATIONS

Apart from the commonly known, the following abbreviations are used in this thesis:

ACE	: angiotensin I converting enzyme
ADH	: antidiuretic hormone
AI/II	: angiotensin I/II
bpm	: beats per minute
BRS	: baroreflex sensitivity
bw	: body weight
CCO	: common carotid occlusion
CEBs	: calcium entry blockers
CI	: cardiac index
CNS	: central nervous system
CO	: cardiac output
CSP	: carotid sinus pressure
DA	: dopamine
ERPF	: effective renal plasma flow
FF	: filtration fraction
GFR	: glomerular filtration rate
HP	: heart period
HQF/R	: hindquarter flow/resistance
HR	: heart rate
kw	: kidney weight
MAP	: mean arterial pressure
MF/R	: mesenteric flow/resistance
PAH	: para amino hippuric acid
PEG	: poly ethylene glycol
PMSF	: phenyl methyl sulfonyl fluoride
PRC	: plasma renin concentration
RF/R	: renal flow/resistance
SAD	: sino-aortic baroreceptor denervated
SHR	: spontaneously hypertensive rat(s)
SV(I)	: stroke volume (index)
TPR(I)	: total peripheral resistance (index)
$U_{Na}V$	: urinary sodium excretion
V^{Na}	: urine production
WKY	: Wistar-Kyoto

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Hypertension affects 15-20% of all adults in western civilisation. It is an important risk factor for cardiovascular complications. The major long-term hemodynamic change in essential hypertension is an increase in total peripheral resistance. In the circulation, total peripheral resistance is determined by the resistances of parallel vascular beds:

$$R = \left(\frac{1}{R_1} + \frac{1}{R_2} + \frac{1}{R_3}\right)^{-1}$$

in which R_1, R_2, etc. each are resistances of individual vascular beds. At the level of a single blood vessel, the resistance can be approximated by Poiseuille's law for flow in a straight tube:

$$R = \frac{8\rho L}{\pi r^4}$$

in which r is the radius and L the length of the tube; ρ is the viscosity of the blood, and π is a proportionality constant. For the resistance of a whole vascular bed, the total number of blood vessels is an additional factor of importance.

It has been suggested that a decrease of the number of perfused blood vessels is a mechanism by which total peripheral resistance can increase in hypertension (Hutchins et al, 1974; Henrich et al, 1978; review Bohlen, 1985b). However, from Poiseuille's law the most important factor which determines the resistance seems to be the radius of

the vessel. Most hypotheses on the pathogenesis of hypertension, therefore, focus on a possibly decreased lumen of resistance vessels.

These hypotheses can be distinguished on the basis of factors leading to a decreased lumen. In a first hypothesis, the decrease of the lumen is considered as a primary mechanism causing hypertension. Several authors have proposed that in this case the decreased lumen of arterioles is the consequence of increased sensitivity of blood vessels to pressor transmitters or hormones (Case et al, 1977; Phillipp et al, 1978; Weber et al, 1981; Johnston et al, 1981; Abboud, 1982) or a deficiency of endogenous vasodilator substances (Lazarus et al, 1974; McGiff et al, 1981; Furchgott and Zawadzki, 1980). Other hypotheses explain the decreased lumen as secondary to the hypertension. Folkow (1978) suggested a generalized wall hypertrophy. A third hypothesis is the so-called autoregulation concept, originally developed by Borst and Borst-De Geus (1963) and Guyton et al (1972). In that hypothesis, the rise in peripheral resistance is the ultimate consequence of a chain of events - explained in more detail in paragraph 1.2 - that is triggered by a change in the renal excretion of fluid.

These different approaches to the mechanism of increased resistance in hypertension have not only important pathogenetic, but also therapeutic implications. The mechanisms whereby an optimal reduction of peripheral resistance is achieved may differ depending upon proposed mechanism. This thesis is an attempt to analyze the regional hemodynamic effects of antihypertensives from this point of view. A special emphasis will be given to the third of the above mentioned mechanisms: the long-term elevation of blood pressure and peripheral resistance is the consequence of a renal deficiency in sodium and water excretion. In the rest of this introductory chapter, the role of the kidney in the development of hypertension shall be briefly discussed first. Then, the role of renal perfusion in sodium and water excretion is reviewed. This physiological part will then be followed by an introduction into the regional hemodynamics of antihypertensive drugs with a special emphasis on their renal effects.

1.2 The role of the kidney in the development of hypertension

Several investigators have focussed on an important role of the kidney in the initiation of the increase in vascular resistance as observed in essential hypertension. Traube (1871) was one of the first investigators who suggested that hypertension might be a consequence of impaired renal excretory function. However, the quantitative importance of renal excretory function in regulating blood pressure remained rather vague until the 1960s when Borst and Borst-De Geus (1963) began to develop the concept that long-term blood pressure is dictated primarily by renal excretory function. This concept was elaborated by Guyton (1974) who stressed the importance of the relationship beween blood pressure and urine excretion, the so-called renal function curve. Under normal circumstances, a small increase or decrease in blood pressure leads to a large increase or decrease in urine excretion. If for any reason, urine excretion is decreased, blood pressure will go up and the increased blood pressure restores urine output at the expense of hypertension. Such a shift of the renal function curve to a higher blood pressure has indeed been observed in experimental hypertension (Norman et al, 1978; Roman and Cowley, 1985).

Experimental evidence for the involvement of the kidney in the development of hypertension was obtained by several investigators. Bianchi et al (1973, 1977) and Dahl and Herne (1973) showed that if kidneys from Milanese and Dahl-hypertensive rats respectively were transplanted into normotensive rats, these latter animals developed hypertension even when the transplanted kidneys were from animals of which the blood pressure had not yet increased. Similar results were observed by Kawabe et al (1978) in spontaneously hypertensive rats.

Several authors have suggested an involvement of the sympathetic nervous system in the change in renal function. Not only do the renal sympathetic nerves control renal vasoconstriction and release of the pressor hormone renin, but also tubular sodium reabsorption depends upon renal sympathetic nerve activity. Application of noradrenaline to the perfusate of isolated rat kidneys increases sodium reabsorption when perfusion pressure was held constant (Besarab et al, 1977).

Furthermore, in isolated proximal tubules of the rat, noradrenaline was able to stimulate fluid reabsorption when applied from the peritubular but not from the luminal side (Bello-Reus, 1980; Chan, 1980). Electrical stimulation of renal nerves in anesthetized rats, at an intensity that does not affect renal blood flow or glomerular filtration rate, decreases urinary flow and sodium excretion through an increase in reabsorption at the proximal tubular level (DiBona and Sawin, 1982).

These studies suggest that renal nerves may be involved in a mechanism by which the renal function curve can shift to a higher blood pressure level. In fact, it has been shown that elimination of the sympathetic innervation of the kidney by surgical denervation at least retards the development of genetic hypertension (Liard, 1977; Kline et al, 1978; Diz et al, 1982) and DOCA-salt hypertension (Katholi, 1980). Renal denervation also reduces the arterial pressure levels in Goldblatt-hypertensive rats and in dogs (Katholi et al, 1981, 1982). Katholi et al (1981, 1982) suggested from studies in several hypertensive animal models that afferent renal nerves may play an important role in the maintenance of hypertension via a direct feedback mechanism influencing efferent sympathetic tone. This effect on efferent sympathetic tone leads then to an increase in vascular resistance, sodium and water retention and renin release.

Borst and Borst-de Geus (1963) and Guyton (1974) suggested that impairment of renal function is accompanied by sodium and water retention leading to expansion of intravascular volume. This increases cardiac output and causes overperfusion of several tissues. This overperfusion is then opposed by local autoregulatory mechanisms which prevent the tissues from further overperfusion by increasing their vascular resistance, and thus increase systemic blood pressure.

All the evidence discussed thus far implies a renal retention of sodium and fluid as a prerequisite for the development of hypertension. Investigators, however, acknowledge that expansion of fluid volume has not been found in all hypertensive animal models (Haddy et al, 1979).

A more recent hypothesis proposes a primary role for a natriuretic hormone which is hypothalamic in origin and is a potent inhibi-

tor of the ouabain-sensitive Na^+/K^+ ATPase (De Wardener et al, 1981; Pamnani et al, 1981; Huot et al, 1983; Haddy et al, 1985). Inhibition of the Na^+ pump leads to an increased intracellular Na^+ and Ca^{2+} uptake and thereby increases vascular contractility and reactivity (Blaustein, 1974; De Wardener et al, 1981; Haddy et al, 1985) and hence vascular resistance.

De Wardener et al (1983) and Gruber et al (1980) reported that expansion of fluid volume in dogs indeed releases a natriuretic hormone. However, Jandhyala and Ansari (1986) and Beasly and Malvin (1985) have shown that not volume expansion but elevation of cerebrospinal fluid Na^+ levels triggers the release of a natriuretic hormone, perhaps by activation of Na^+ sensitive and/or osmosensitive sites. The same stimulus in the CNS releases antidiuretic hormone (ADH). So, a combined renal action of ADH and natriuretic hormone may prevent volume expansion. This suggests that the primary stimulus for the development of hypertension may be the cerebrospinal fluid Na^+ levels and not necessarily fluid expansion. Thus far, this natriuretic factor has not been isolated, so its existence is still uncertain.

In summary, the above mentioned hypotheses suggest that the primary mechanism which causes hypertension is a decrease in sodium excretion as a consequence of a renal defect. A causal approach for the treatment of hypertension would, therefore, be an enhancement of the renal capacity to excrete sodium. Such an approach is available since many years in the form of diuretics. These drugs reduce tubular sodium reabsorption and reduce blood pressure. These drugs increase, however, the renin release without tachyphylaxis. This leads to counterregulation of the blood pressure reduction and limits their antihypertensive ability (Vaughan et al, 1978).

In section 1.3, another possible way by which an increase in sodium excretion could be induced, viz. via a change in renal perfusion, is presented.

1.3 Renal perfusion and sodium excretion

Total renal sodium excretion is the difference between the

quantities filtered in the glomeruli and reabsorbed in the tubuli. The glomerular filtration rate is determined by the glomerular and tubular hydrostatic and the counteracting oncotic pressure, and the capillary filtration coefficient of the glomerulus. In rats the glomerular flow has strong influence on the glomerular filtration rate (Marchand and Mohrman, 1980) which is in contrast to the situation in dogs where the effective glomerular filtration pressure strongly influences glomerular filtration rate. This was concluded from micropuncture studies, in which measurements in various parts of the nephron showed that there is a filtration equilibrium in the rat (Blantz, 1977; Marchand and Mohrman, 1980) but not in the dog (Ott and Marchand, 1976; Marchand and Mohrman, 1980), i.e. in the rat the glomerular hydrostatic pressure and oncotic pressure cancel each other somewhere at the glomerular membrane whereas in the dog oncotic pressure is always lower. From other species (including man), these data are not available and it is not known whether there is a filtration equilibrium.

Glomerular blood flow is determined by the blood pressure in the whole arterial circulation and afferent and efferent renal vascular resistance.

Renal vasodilators may dilate afferent and/or efferent arterioles. It depends on where the particular renal vasodilator has its most pronounced effect to what degree GFR may be influenced. However, most substances used so far to cause renal vasodilation (acetylcholine, bradykinin and prostaglandins) increase renal blood flow and sodium excretion without affecting glomerular filtration rate (Marchand et al, 1977; Hartupee et al, 1982; Haas et al,1984; Mertz et al, 1984). These studies indicate that, apart from an increase in GFR, a reduction in tubular reabsorption of sodium can be the cause of natriuresis after renal vasodilation.

Early and Friedler (1965) and later Fadem et al (1980) postulated that the natriuresis is a consequence of a washout of the solute gradient of the medullary interstitium induced by increased renal plasma flow. However, Hartupee et al (1982) showed that a medullary gradient washout was not accompanied by a natriuresis when renal interstitial hydrostatic pressure was controlled. These results suggest that an increase in renal interstitial hydrostatic pressure is an

essential component of the natriuresis associated with renal vasodilation. Marchand et al (1977), Hartupee et al (1982), Haas et al (1984), and Mertz et al (1984) also measured peritubular capillary hydrostatic pressure. They observed an increase in peritubular capillary pressure upon renal vasodilation. Marchand et al (1977) compared the effects of the renal vasodilators secretin and acetylcholine on renal interstitial hydrostatic pressure and sodium excretion in dogs. Whereas both vasodilators increased renal blood flow and peritubular capillary hydrostatic pressure similarly, only acetylcholine increased interstitial hydrostatic pressure and sodium excretion. Haas et al (1984) compared the effects of intrarenal infusion of prostaglandins on sodium excretion. They showed that when renal interstitial hydrostatic pressure was allowed to increase during infusion of prostaglandin E_2 there was a marked increase in fractional excretion of sodium. However, when the rise in renal interstitial hydrostatic pressure was prevented by prior renal decapsulation and a slight reduction in renal perfusion pressure via aortic constriction the natriuresis was markedly attenuated despite similar increases in renal blood flow. Similar results were observed by Hartupee et al (1982). Taken together these studies suggest that renal interstitial hydrostatic pressure is the crucial factor linking renal hemodynamics to sodium excretion.

In conclusion, the above mentioned observations suggest that selective renal vasodilation could be a way to increase sodium excretion by reducing tubular sodium reabsorption without inhibiting the Na^+/K^+ pump. The mechanism by which the renal vasodilators induce natriuresis is not clear. However, an increase in interstitial hydrostatic pressure seems to be essential.

1.4 Vasodilators versus renal vasodilation

The primary pharmacological effect of arteriolar vasodilators is a relaxation of vascular smooth muscle. This leads to a fall in peripheral resistance and blood pressure. This causes an increase of venous return, and a baroreflex-mediated increase in heart rate and

cardiac output. A second relatively rapid response to vasodilator therapy is an increase in renin release. This rise in renin release is caused by the increased sympathetic discharge to the kidney and reduced renal perfusion pressure. A third physiological response to vasodilator therapy is the retention of sodium and water due to the decreased renal perfusion pressure and increased renin activity. This retention of sodium and water causes an expansion of extracellular and blood volume.

In pharmacotherapy, most directly acting vasodilators are usually given as a third step after beta-adrenoceptor blocking drugs and/or diuretics which is related to the above mentioned less favorable actions, during single use of these drugs. (Koch-Weser, 1974 ; Gross, 1977).

Computer simulation of an arteriolar vasodilation, using the mathematical model of the cardiovascular system previously published by Guyton et al (1972) predicts short-term changes quite similar to those observed experimentally (Struyker Boudier, 1980), e.g. a rapid fall in total peripheral resistance, arterial pressure and urinary excretion rate, an early increase in sympathetic nerve activity, cardiac output and extracellular fluid volume. Moreover, the simulation predicts that long-term effects include a continuous reduction of blood pressure and total peripheral resistance and normalization of sympathetic nerve activity and urinary excretion rate, whereas cardiac output and extracellular fluid volume remain elevated.

Similar computer simulation studies (Struyker Boudier, 1980) predict that a drug with preferential renal vasodilating properties could be a candidate for an optimal treatment of hypertension. Fig. 1.1 shows a simulation of the effects of a drug dilating the afferent (preglomerular) arterioles of the kidney starting at the point indicated by the arrow. The results of the simulation indicate an immediate rapid diuresis leading to a slight reduction of extracellular and blood volume as well as a slight decrease in cardiac output. In the course of several days, blood pressure drops gradually below predrug level. This reduction in blood pressure is parallelled by a gradual drop in total peripheral resistance, a restoration of cardiac output, excretion of water and salt and blood volume to values close

to their predrug levels. Extracellular fluid volume remains slightly diminished. Sympathetic nerve activity is elevated to a slight degree during the first few days of renal vasodilator therapy but returns to normal values shortly thereafter. Moreover, the activity of the renin-angiotensin-aldosteron system is not influenced significantly during the chronic phase of renal vasodilation therapy.

These simulated effects of a preferential renal vasodilation can be explained by reversing the hypothesis of Borst and Borst-De Geus

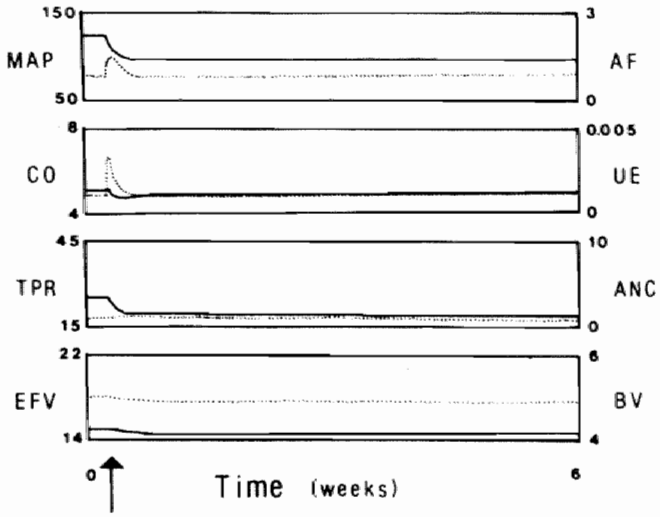


Fig. 1.1: Computer simulation of the effects of a selective renal vasodilator in a hypertensive patient. At the point indicated by the arrow, the resistance of the afferent (preglomerular) arterioles of the kidney was reduced. The solid lines correspond to parameters on the left side of each panal; the dotted lines to parameters on the right. The total duration of the simulation period was six weeks. MAP: mean arterial blood pressure (mm Hg); CO: cardiac output (l/min); TPR: total peripheral resistance (mm Hg/l/min); EFV: extracellular fluid volume (l); AF: autonomic factor, i.e. overall activity of the autonomic nervous system (ratio to a normal value of 1: an increase from 1 represents an increase in sympathetic activity or a decrease in parasympathetic activity); UE: urine excretion (l/min); ANC: relative angiotensin concentration in plasma (ratio to normal value of 1); BV: blood volume (l).

(1963) and Guyton (1974) for the development of hypertension. The renal vasodilation is accompanied by sodium and water excretion leading to a reduction of intravascular blood volume and extracellular fluid volume. This decreases cardiac output and perfusion of several tissues. This reduced perfusion is then opposed by local autoregulatory mechanisms which prevent the tissue from further underperfusion by decreasing their vascular resistance and thus blood pressure. This reduction in vascular resistance leads then to an increase in venous return which consequently increases cardiac output close to normal. The decreased blood pressure restores urine output at a lower blood pressure. So, the result would be a shifting of the kidney function curve to a lower blood pressure.

The results of the computer simulation suggest that the reduction of blood pressure is achieved with a minimum of disturbances of other hemodynamics and endocrine parameters, except for total peripheral resistance which drops in parallel to the fall in blood pressure suggesting that renal vasodilation is an optimal therapy for the treatment of hypertension.

The above presented hypothesis has so far not been investigated experimentally. Thus the question is raised whether there are substances with a preferential renal vasodilation and whether they increase sodium and water excretion. In the next part of this chapter the effects of several antihypertensives on renal vascular resistance and sodium and water excretion shall be reviewed.

1.5 Regional hemodynamics of antihypertensive drugs with a special emphasis on renal effects

In this section, the regional hemodynamics of several antihypertensive agents shall be reviewed with a special emphasis on the effects on renal vascular resistance and sodium and water excretion.

1.5.1 Directly acting vasodilators

The effects of different directly acting vasodilators on renal blood flow and vascular resistance have been assessed in clinical research using clearance techniques. Hydralazine causes increased

renal blood flow unlike minoxidil or diazoxide (Reubi, 1950; Johnson, 1971; Zins, 1974; Leiter et al, 1981). Also in conscious dogs Chelly et al (1986) observed a preferential renal vasodilation after hydralazine. However, Bolt and Saxena (1984) found a more general vasodilation in hypertensive rabbits after hydralazine. They observed not only a reduction in vascular resistance in the kidney but also in other vascular beds, e.g. heart, brain, and large intestine, after a single dose of hydralazine.

When comparing the above described regional hemodynamic effects with those in the studies of Gottlieb et al (1972) and Hanamer et al (1971) it seems unlikely that directly acting vasodilators preferentially dilate the renal vascular bed since they cause a retention of sodium and water and an increase in renin release from the kidney (Gross, 1977). The general vasodilation strongly reduces systemic blood pressure and consequently renal perfusion pressure. This decrease in renal perfusion pressure may then be responsible for the observed sodium and water retention and the increase in renin release.

1.5.2 Calcium entry blockers

Of the available studies, only a few show renal vasodilating properties of calcium entry blockers (CEBs). Drextler et al (1985) and Kanda and Flaim (1984) found a reduction in renal resistance in conscious normotensive rats after a single dose of different CEBs. However, this was not a preferential renal vasodilation. These investigators observed also a reduction in vascular resistance in other vascular beds. Drextler et al (1985) found that nisoldipine also reduces coronary and skeletal muscle vascular resistance. Kanda and Flaim (1984) reported a reduction of resistance in coronary, skeletal muscle and mesenteric vascular beds after nifedipine.

In other regional hemodynamic studies, no renal vasodilation was found following CEBs in several species. Flaim and Zelis (1982) showed that diltiazem reduces resistance in muscular, coronary, gastrointestinal and cerebral vascular beds in normotensive conscious rats. Skin and kidney did not dilate. Reed and Tuma (1986) observed for nifedipine in anesthetized normotensive rats a general vasodilation with a most pronounced effect in skeletal muscle. Sesoko et al (1984) repor-

ted that nitrendipine was potent as a vasodilator in most organs except skin and skeletal muscle in normotensive conscious rats. In anesthetized cats, most CEBs reduce vascular resistance in the muscular bed (Hof et al, 1982). However, this effect was more pronounced after the dihydropyridine derivatives nifedipine, nicardipine and PY 108-068 than after verapamil or diltiazem. Hof and co-workers (1983) also observed a consistent reduction in cerebral and coronary resistance whereas skin and renal resistances remained unchanged in normotensive anesthetized rats. The effects on the gastro-intestinal bed were more variable showing a vasodilation after nicardipine and diltiazem and little effect following verapamil and PY 108-068 (Hof, 1983).

In most of the above mentioned regional hemodynamic studies flow changes were measured in normotensive animals using the microsphere technique. However, the directional pulsed Doppler system was used by Barron et al (1983) and Knight et al (1984) to measure regional hemodynamic effects of CEBs in normotensive conscious rats. Barron et al (1983) found that skeletal muscle and, to a lesser degree, mesenteric resistances decreased following nitrendipine, nisoldipine and verapamil. No effect on renal resistance was observed. Knight et al (1984) found a pronounced reduction in vascular resistance in the muscular and mesenteric vascular bed but only a slight reduction in renal resistance.

Taken together, these regional hemodynamic studies suggest that the muscular vascular bed is the preferential site of action for CEBs followed by the coronary and cerebral vascular bed. In other beds, the vascular effects of CEBs are more divergent.

On the basis of the more general vasodilating action of CEBs one would expect a sodium and water retention as a consequence of the reduced renal perfusion pressure. Leonetti et al (1982) reported that nifedipine induced a marked increase in urine volume and sodium excretion in hypertensive patients with a much smaller change in normotensives. Verapamil did not influence water and sodium excretion in either direction. Yokoyama et al (1983) found increases in urinary volume and sodium excretion after nifedipine treatment in patients with essential hypertension. Nordlander et al (1985) observed in

hypertensive rats an increase in sodium and water excretion after nifedipine and felodipine. Also, nicardipine (Chaignon et al, 1986) and nitrendipine (personal communication, Van Zwieten) cause natriuresis in man. In view of the lack of a renal vasodilator effect of CEBs the most likely explanation for this diuretic and natriuretic action is a tubular mechanism either directly or indirectly through an effect on aldosteron (Loutzenhiser et al, 1985).

1.5.3 Beta-blockers

The acute hemodynamic effects of most beta-blockers in hypertensive patients or animals consist of a fall in cardiac output whereas a rise in total peripheral resistance prevents an early fall in blood pressure (Ulrych et al, 1968; Tarazi and Dustan, 1972; Smits et al, 1982; Van Baak et al, 1982; Cofler et al, 1984). Little is known about the regional vascular beds involved in this total peripheral resistance increase.

Hatzinikolaou et al (1983) found an increase in resistance in most vascular beds after propranolol in normotensive Wistar rats using the microsphere method. However, in brain and muscle, vascular resistance was not significantly affected. Van Boom and Saxena (1983) observed an increase in heart, brain, stomach, mesenteric, pancreas, spleen, and skin vascular resistance after propranolol infusion in normotensive rabbits. In renal hypertensive rabbits these investigators found an increase in vascular resistance only in heart and pancreas after propranolol. On the other hand, Nies et al (1973) found a generalized increase in resistance in all vascular beds studied using a propranolol infusion in conscious normotensive monkeys.

These regional hemodynamic studies showed a very divergent pattern during acute beta-blockade with respect to the renal effects. However, in renal hemodynamic studies in dogs (Nies et al, 1971; Nomura et al, 1978), SHR (Smits et al, 1982) and hypertensive man (Weber and Drayer, 1980; Wilkinson, 1982; Epstein and Oster, 1982; Bernstein and O'Connor, 1984) all classes of beta-blockers cause a reduction in renal plasma flow with the exception of nadolol (Duchin et al, 1978; Hollenberg, 1979; Epstein and Oster, 1982) and tertatolol (Laubie et al, 1986; Paillard et al, 1986).

In contrast to the renal flow reduction seen in most studies after beta-blockade, several studies showed an immediate short lasting diuresis and natriuresis for different species following propranolol (Epstein and Oster, 1982; Carrara and Baines, 1976; Shibouta et al, 1979; Smits et al, 1982). Metoprolol also causes this effect which makes it likely that the phenomenon is related to $beta_1$-receptor blockade (Wikstrand, 1983). Smits et al (1982) have suggested that beta-blocker-mediated diuretic and natriuretic effects in the SHR are caused by a tubular action of beta-blockers.

The most direct way in which beta-blockers may interact with renal tubular processes is through interference with sympathetic innervation of these structures. Radioligand binding studies in the rat (Gavendo et al, 1980; Perrot et al, 1984; Struyker Boudier et al, 1986) have shown the existence of $beta_1$-adrenoceptors in tubular fractions of kidney homogenates. Functional evidence for the involvement of tubular beta-receptors in the regulation of sodium excretion comes from studies in which isolated kidneys (Besarab et al, 1977) or tubules (Bello Reus, 1980) were perfused. In these studies, noradrenaline caused a reduction of sodium fluxes, whereas this effect could be inhibited by propranolol. These experiments suggest that beta-blockers may directly interact with tubular function. This causes an effect which cancels the expected water and sodium retention as a consequence of the reduced renal perfusion.

1.5.4 Angiotensin I converting enzyme inhibitors

A possible preferential renal vasodilator activity of angiotensin I converting enzyme (ACE) inhibitors has been described in hypertensive patients (Wong et al, 1981), normotensive and renal hypertensive dogs (Zimmerman et al, 1980; Oliver et al, 1983) and spontaneously hypertensive rats (Richer et al, 1983; Smits and Struyker Boudier, 1984). It is not clear whether the reduction of formation of angiotensin II is solely responsible for the decrease in resistance because the ACE inhibitor captopril also has an antihypertensive effect in patients with low plasma renin levels (Schalekamp, 1984; Man in 't Veld et al, 1980). There is evidence that prostaglandins may be involved in captopril's antihypertensive effect because increased levels of

plasma and urinary metabolites of prostacyclin have been detected and furthermore no antihypertensive effect of captopril was observed after prostacyclin synthesis inhibition (Mimran et al, 1980; Fouad et al, 1980; Swartz et al, 1980; Goldstone et al, 1981; Hornych et al, 1982).

Another possibility could be an involvement of bradykinin. ACE is identical to kininase II which is responsible for the degradation of bradykinin, a potent vasodilator. So inhibition of this enzyme may increase plasma bradykinin and induce vasodilation.

It is not yet clear why ACE inhibition is associated with a preferential renal vasodilator effect. Possibly there are differences in the degree of local ACE inhibition in different tissues (Cohen et al, 1982, 1983) or these drugs are bound to renal tissue more than elsewhere in the body (Drummer et al, 1983).

Several studies have shown that captopril and enalapril increase or have no effect on sodium and water excretion, whereas a blood pressure reduction is observed (Zimmerman et al, 1980; Wong et al, 1981; DeBruyn et al, 1981; Hollenberg, 1982; Oliver et al, 1983; Smits and Struyker Boudier, 1984). Some of these investigators suggested that the reduction in angiotensin II and aldosteron formation causes these renal excretory effects and not the marked renal vasodilator effect of captopril and enalapril.

1.5.5 Prostaglandins

Several naturally occurring prostaglandins, e.g. PGE_2 and PGI_2 (prostacyclin) are well known for their renal vasodilator effect upon renal administration (Strandhoy et al, 1974; Hockel and Cowley, 1979; Bear and McGiff, 1979; Imanishi et al, 1980). Blaine et al (1982) described the synthesis of an orally active prostaglandin analogue 4-(3-(3-(2-(1-hydroxycyclohexyl)ethyl)-4-oxo-2-thiazolidinyl)propyl) benzoic acid. They showed that in conscious dogs with chronically implanted electromagnetic flow probes, oral administration of this agent causes a dose-dependent increase in renal but not in lower aortic blood flow. No natriuresis was observed in this study.

Several studies showed that prostaglandins influence renal excretory function. When intrarenal infusion of prostaglandin E_2 was performed in anesthetized dogs, Johnston et al (1967) noted that urine

volume, urine sodium excretion, free water clearance and renal plasma flow increased. In this study, glomerular filtration rate and mean arterial pressure remained unchanged. Similar changes following PGE_2 infusion have been observed by Martinez-Maldonado et al (1972), Gross et al (1973), Strandhoy et al (1974) and Arendshorst et al (1974). Haas et al (1982) observed an increase in sodium excretion after PGE_2 in rats. Hockel and Cowley (1979) observed in dogs during long-term intra-renal PGE_2 infusion a chronic diuresis whereas sodium excretion was only increased during the first hours. They also observed a 10-fold elevation in plasma renin activity. Studies performed by Yun et al (1977) and Gerber et al (1978) indicated that increases in plasma renin activity may be induced by a direct prostaglandin effect on the juxtaglomerular cells.

1.5.6 Dopamine

Another group of potential renoselective vasodilators are the dopamine analogs. Studies by Goldberg et al (1978) indicate the presence of dopamine receptors in the renal vasculature, stimulation of which causes renal vasodilation. On the basis of this observation a number of dopamine analogs have been tested for their renal vasodilator potential (Pendleton et al, 1978; Hahn et al, 1982; Ackerman et al, 1983).

Goldberg and Kohli (1979, 1983) have proposed a classification of dopamine receptors into DA_1 and DA_2 receptors. The DA_1 receptor is mostly located on vascular smooth muscles in the kidney, whereas DA_2 receptor are located on sympathetic nerve terminals and autonomic ganglia (Struyker Boudier, 1986).

Antihypertensive properties of the dopamine DA_1 receptor agonist fenoldopam have been described in both experimental animals (Ackerman et al, 1982; Berkowitz and Ohlstein, 1984) and humans (Ventura et al, 1984; Redman et al, 1985; Cregeen et al, 1985). It was claimed initially that fenoldopam due to its predominant DA_1 activity would be a vasodilator with selectivity for the kidney (Ackerman et al, 1982). Later studies have indicated that it is a more general vasodilator (Ackerman et al, 1983; Lappe et al, 1984; Redman et al, 1985).

Dopamine causes diuresis and natriuresis in different mammalian

species independent of its renal hemodynamic effects. A tubular site of action seems responsible for this dopamine effect (Goto et al, 1979; Wassermann et al, 1980). Ackerman et al (1983) found also for fenoldopam a natriuretic activity in different species.

1.5.7 Alternative approaches

The above mentioned preferential renal vasodilation results from pharmacodynamic selectivity of the substances. Another possible design of drugs with preferential renal vasodilator activity based on a selective kinetic action could be the development of renal prodrugs. A prodrug is an agent that is biologically inactive in itself, but contains an active drug that is formed only after metabolic conversion. By using enzymatic routes, specific for a certain organ, one can achieve site-selective formation of the active drug (Smits and Thijssen, 1986). This principle has already been used successfully in the design of selective dopamine formation from gammaglutamyl-L-dopa in the treatment of acute renal failure in rats (Casson et al, 1983). Hofbauer et al (1985) reported the pharmacology of the renal prodrug CGP 22 979A and its active compound CGP 18 137A which is to be regarded as a hydralazine-like vasodilator. Sequential hydrolysis by acylase and glutamyltranspeptidase is needed in order to generate the active substance. These reactions occur in the kidney at a higher rate than in other tissues (Orlowski et al, 1980). Renal vasodilator prodrugs may offer an alternative approach to the question of selective renal vasodilatation as a mechanism of treating hypertension. In the later chapters of this thesis, this approach will be discussed in more detail.

1.6 Aim of the present investigations

In a previous part of this chapter the possible advantage of preferential renal vasodilation over other antihypertensives in the treatment of hypertension was discussed. Most antihypertensive drugs induce intrinsic side-effects, which limits the antihypertensive potential of these drugs and forces the use of combination therapy. So

far, the above presented hypothesis for antihypertensive action of selective renal vasodilators has not been investigated experimentally.

Therefore, the studies described in this thesis were designed to investigate the regional hemodynamic effects of antihypertensive drugs with a special emphasis on their renal effects. The ultimate goal of this thesis was to investigate whether selective renal vasodilation could cause long-term antihypertensive effects. This hypothesis was investigated with a renal vasodilator prodrug. The effects of this compound were compared with the central and regional hemodynamic effects of other antihypertensives, i.e. directly acting vasodilators, calcium entry blockers and beta-adrenoceptor blockers. A first requirement for this analysis is the use of a conscious hypertensive animal model. The spontaneously hypertensive rat was chosen for this purpose. Chapter 2 describes the experimental techniques used for hemodynamic and renal function studies in conscious rats.

In order to fully understand the mechanisms of action of any antihypertensive drug a second requirement is a careful analysis of its interaction with the mechanisms involved in blood pressure regulation. The most important blood pressure regulating mechanism in the acute situation is the sino-aortic baroreceptor reflex. The role of the baroreceptor reflex was determined in extended studies by comparing central and regional hemodynamics of several antihypertensives in intact and baroreceptor denervated animals.

For a better explanation of these effects the influence of a non-pharmacological baroreceptor reflex activation (unilateral common carotid occlusion) on regional hemodynamics was determined and described in chapter 3.

Subsequently, chapters 4, 5 and 6 describe the results of a number of studies on the acute as well as long-term hemodynamic effects of classical vasodilators, calcium entry blockers and beta-adrenoceptor blockers. Furthermore, the influence of the baroreflex on these effects was assessed in each case. In a number of studies additional measurements of renal function and plasma renin concentration were made. Finally, in chapter 7 a series of studies is presented on the acute and long-term hemodynamic and renal effects of a new renal vasodilator prodrug.

CHAPTER 2

MATERIALS AND METHODS

2.1 Animals

Male normotensive Wistar-Kyoto (WKY) and male spontaneously hypertensive rats (SHR), aged 10-16 weeks and weighing between 250-350 g, were used in the studies described in this thesis.

The WKY rats were obtained from the original WKY strain and the SHR from the original SHR strain bred at the Central Animal Facilities of the University of Limburg, Maastricht, The Netherlands.

Before the experiments, animals had free access to standard lab food (Hope Farms, type RMH-TM, Woerden, The Netherlands) and tap water. After surgery, the rats were housed separately. The conditions during the experiments are described in the experimental protocols.

2.2 Catheters and surgery

In the experiments described in the following chapters four types of catheters were implanted for different purposes.

2.2.1 Abdominal aorta catheter

This type of catheter was developed for direct measurements of blood pressure in conscious rats. In the renal hemodynamic studies, this catheter was used for blood sampling.

The catheter was constructed from a 9-cm piece of PE-10 tubing, heat-sealed to a 12-cm piece of PE-50 tubing. Two small rims were made at the connection to facilitate securing the catheter. On the other

end of the PE-50 tubing, a 1-cm piece of PE-100 tubing was heatsealed. Thereafter, a 3-cm piece of vinyl tubing (Serva TT63) was slid over the 1 cm PE-100 tubing. Finally, the PE-10 tubing was bent in a J-shape by dipping it into near-boiling water.

The abdominal aorta was cannulated by an approach through the right femoral artery. Under light ether anesthesia, a small incision was made in the right groin and the femoral artery was freed from connective tissue. After clamping it, a small hole was cut in the vessel and the PE-10 catheter was inserted. It was advanced for 3-4 cm into the abdominal aorta. The catheter was secured to the artery and to the leg muscle with silk and guided subcutaneously to the neck where it was exteriorized. The catheter was filled with heparinized saline (50 IU/ml in 0.9% NaCl) and closed with a metal plug. The catheter was implanted at least one day before the experiments.

2.2.2 Vena cava catheter

This type of catheter was used for administration of bolus injections and infusions in the acute studies in conscious, freely moving rats. The venous catheter was constructed of a 9-cm piece of PE-10 tubing, sealed to a 15-cm piece of PE-50 tubing. Two small rims were made at the connection and 12 cm further for suturing the catheter to the leg respectively neck muscle.

Under light ether anesthesia, a small incision was made in the right groin and the femoral vein was dissected from the surrounding tissue and clamped with two silk sutures. A small hole was cut in the vessel and the venous catheter was advanced for 4 cm into the vena cava. After filling the catheter with heparinized saline (50 IU/ml in 0.9% NaCl), it was closed with a metal plug. The cannulation was performed at least one day before the start of the experiment.

2.2.3 Jugular vein catheter

This type of catheter was used for long-term infusions in combination with the Alzet™ osmotic minipump (Smits, 1980). The catheter was constructed of a 10-cm piece of Silastic ™ medical grade tubing (Dow-Corning 602-155). The proximal end was closed with Silastic™ medical adhesive silicon type A (Dow-Corning 891) and the same mate-

rial was used to make two small rims in the middle of the tubing. After a drying period of 24 hr, the catheter was punctured with a 0.6 mm needle 8 mm from the silicon-closed proximal end.

Surgery was performed under light ether anesthesia. Through a 2-cm ventral mid-line incision in the neck the right jugular vein was localized and freed from surrounding tissue. A small hole was cut in it and the plugged end of the catheter was advanced into the vessel for 3 cm. The catheter was fixed to the vessel and the neck muscle. The distal end was passed through the muscles of the back of the neck and exteriorized there. It was filled with heparinized saline and closed with a ligature and stored subcutaneously until the osmotic minipump was connected. The catheter was implanted at least one day before the start of the experiment.

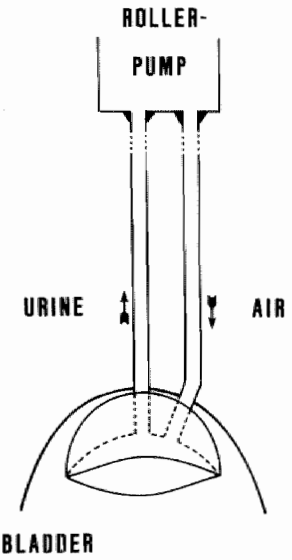


Fig. 2.1: Schematic drawing of the bladder catheter.

2.2.4 Bladder catheter

This type of catheter (see fig 2.1) was used for continuous urine sampling in conscious rats (Daemen et al, 1987). The implant-

ation of the bladder catheter was performed 3-4 days before the experiment. In a small ethylene vinyl acetate co-polymer (EVA) cup (diameter 5.5 mm; removable flow moderator cup from Alzet 2001 osmotic minipump; Alza corporation, Palo Alto, Ca, USA), two holes were made with a hot 0.8 mm needle. PE-50 tubing (25 cm) was inserted into each hole. The tubing fitted snugly in the holes and did not need further fixation to the EVA cup. The catheter system was then sterilized with ethylene oxide. For implantation, a small suprapubic incision was made. The bladder was exteriorized and fixed by clamping it with a small hemostat, the ends of which were covered with silicon tubing. A 6-mm incision was made in the apex of the bladder, taking care not to cut blood vessels. The vinyl cup was inserted through the incision after which the bladder wall was repaired with 6-0 silk sutures and an atraumatic needle. This procedure was carried out under strict aseptic conditions. The PE-50 tubing was then tunneled subcutaneously to the neck where both catheters were exteriorized. The bladder catheter system was carefully flushed with sterile 0.9% NaCl solution, using one of the tubes as an inlet and the other one as an outlet. The catheters were then closed with metal plugs.

2.3 Measurements of central hemodynamics

At least 4 days before the start of central hemodynamic experiments, rats were anesthetized with pentobarbital (60 mg/kg i.p.). The skin was opened over the right third intercostal space and the underlying muscle layers (m. pectoralis profundus and m. rectus abdominus) were cut. After this, the trachea was intubated and artificially ventilated with 60 breaths/min (tidal volume 1.5-3 ml), using a Harvard respiration pump (model 680, Harvard Milles, Mass, USA). Then, the thorax was opened by making a small hole in the intercostal muscle and pleurae using forceps. Thereafter, the intercostal muscle and pleurae were carefully cut over a distance of approximately 15 mm without damaging the lungs. The ribs were spread with a miniature retractor and the aorta was prepared free. An electromagnetic flowprobe with a diameter of 2.4 mm (Skalar, Delft, The Netherlands) was placed around

the ascending aorta at a distance of 3-4 mm from the heart. The probe cable was guided subcutaneously to the neck where the connector was secured to the skin. Details of the procedure have been described elsewhere (Smith and Hutchins, 1979; Smits et al, 1982). At least one day before the start of the experiments, venous and intra-arterial catheters were implanted under light ether anesthesia. A PE-10 catheter for intravenous injections was implanted into the vena cava through the right femoral vein. Arterial blood pressure was measured via PE-10 catheter in the abdominal aorta, through the right femoral artery. Both catheters were guided to the neck where they were exteriorized. After the catheters had been filled with heparinized saline (50 IU/ml) they were closed with metal plugs.

On the morning of the experimental day, the implanted measuring devices were connected to the respective equipment. Arterial blood pressure was measured from the intra-aortic catheter, using a miniature strain-gauge transducer (model CP-01; Century Technology Company, Inglewood, Ca, USA). Blood flow through the ascending aorta was measured with a sine-wave electromagnetic flowmeter (Skalar, model 601, Delft, The Netherlands). Late diastolic flow was taken to be zero and mean blood flow was used as a measure of cardiac output. Heart rate was obtained from either the pressure or the flow signal which were used to trigger a tachograph. Mean signals were obtained by low-pass filtering of the signals.

Cardiac output was normalized for body weight (cardiac index, CI) and expressed as ml/min.100 g body weight. The total peripheral resistance index (TPRI) was calculated from CI and mean arterial pressure (MAP) according to: TPRI = MAP/CI and expressed as mm Hg.min. 100 g body weight/ml. Stroke volume index (SVI) was calculated according to: SVI = CI/HR (HR=heart rate) and expressed as ul/100 g body weight.

2.4 Measurement of regional blood flows

Regional blood flows were measured in separate series of SHR or WKY using a 20-MHz directional pulsed Doppler system with miniaturized

Doppler probes (Haywood et al 1981; Smits and Struyker Boudier, 1984). The abdomen was opened under pentobarbital (60 mg/kg i.p.) anesthesia. Doppler probes were placed around the left renal artery (0.8-0.9 mm), the superior mesenteric artery (0.9 mm) and the abdominal aorta (1.2 mm) distal to the iliolumbar arteries (see fig 2.2). The latter flow consists mainly of blood flow through the skeletal muscle of the hindquarters and will be referred to as hindquarter flow. The abdomen was closed and the probe cables were guided to the neck where they were soldered to a miniature connector which was mounted on the animal's skull with jeweller's screws and dental cement. After this surgery, the animals were allowed 2-4 days for recovery before implantation of arterial and venous catheters according to the procedures described above.

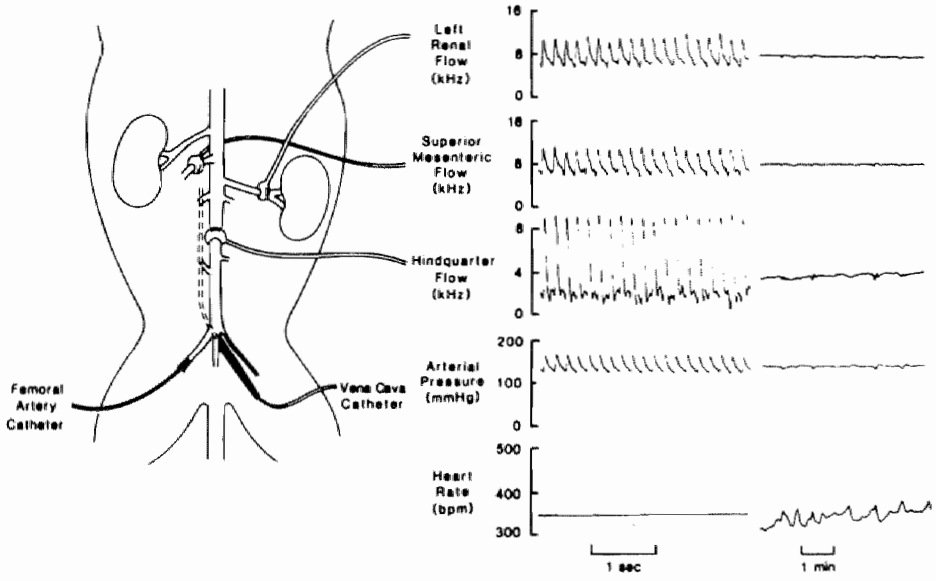


Fig. 2.2: The lay-out of the animal preparations and pulsatile flow tracings of the renal, abdominal, and mesenteric blood flow measured as frequency shifts using a 20-MHz directional pulsed Doppler flowmeter. A catheter in the femoral artery permits simultaneous monitoring of arterial pressure (Haywood et al, 1981).

At least one day after catheter implantation, the animals were connected to the measuring equipment. Regional blood flows were measured as kHz Doppler shift, using a 4-channel 20 MHz directional pulsed Doppler system (Bioengineering Department, University of Iowa City, Ia, USA). It has been documented elsewhere that the Doppler shift is directly and linearly proportional to volume flow (Haywood et al, 1981). Zero blood flow was determined electronically. Mean flows were obtained by low-pass filtering. MAP and HR were measured continuously according to procedures described above. Regional resistance changes (renal resistance: RR; mesenteric resistance: MR; hindquarter resistance: HQR) were calculated from the flow and MAP changes as:

$$\Delta R = \left[\frac{\Delta MAP + 100}{\Delta Flow + 100} - 1 \right] \times 100$$

where all changes are expressed as percentages.

2.5 Baroreceptor denervation

The role of the baroreceptor reflex in the hemodynamic effects of vasodilators was assessed by baroreceptor denervation according to a method previously described (Krieger, 1964; Struyker Boudier et al, 1979). The location of the baroreceptors on the blood vessels together with the main neck nerves are presented in fig 2.3. After exposure of the sheaths enclosing the common carotid arteries, vagi and sympathetic trunks, both vagi and the carotid arteries were freed from the sympathetic trunks. Aortic afferents were interrupted by resecting a 1-cm strip of the sheath and sympathetic trunk. Afferents from the aorta travelling with the recurrent laryngeal nerves were interrupted by resection of the superior laryngeal nerves. The carotid sinus baroreceptors were denervated by stripping the carotid bifurcation and its branches and painting the vessels with 10% phenol in ethanol. This surgery was performed before the implantation of the aortic catheter, leaving at least 2 days for recovery.

The effectiveness of baroreceptor denervation was tested accor-

ding to Struyker Boudier et al (1979) by giving the rats an intra-arterial bolus injection of 5 µg/kg phenylephrine, dissolved in 0.1 ml 0.9% NaCl. This increases mean arterial blood pressure by 35-50 mm Hg. This increase in pressure normally causes a baroreceptor-mediated decrease in heart rate of 40-100 bpm (Struyker Boudier et al, 1979, 1982). Our criterion for effective surgical denervation was that an increase in MAP of 30 mm Hg or more should not cause a fall in heart rate of more than 20 bpm.

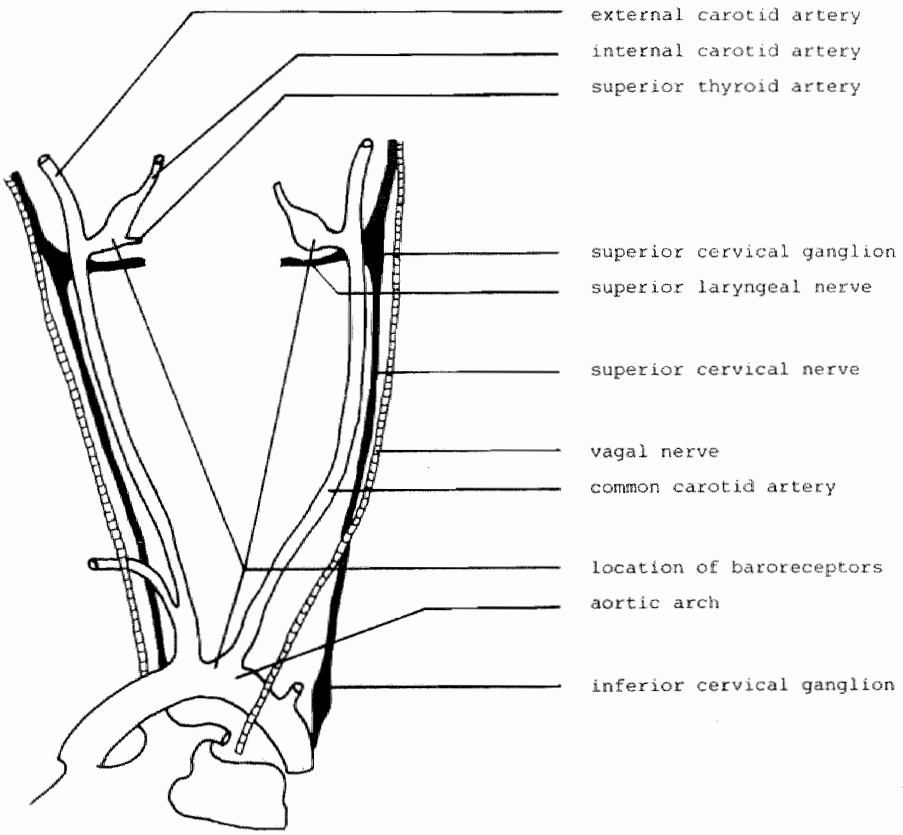


Fig. 2.3: General nerve and blood vessel organisation in the sino-aortic area.

2.6 Baroreceptor unloading

The animals were anesthetized with 10% ketamine (0.2 ml i.m.: Aescoket^{R} Aesculaap) and 2% xylazine (0.1 ml s.c., Rompun^{R}, Bayer), (Krieger, 1964; Struyker Boudier et al, 1979).

A ventral midline incision was made in the neck. The sheaths enclosing the common carotid arteries were exposed and the baroreceptors on the aortic arch and other bifurcation were denervated as described in section 2.5, to avoid a counterregulation by these receptors. Thereafter an occluder and Doppler flowprobe were placed around the right innervated common carotid artery. The occluder was made from a Beckman Dynagraph inkwell tube (inner diameter 0.85 mm outer diameter 1.2 mm), at the end of which an inflatable cuff of about 1.5 cm. This cuff was formed by heating 1.5 cm of the end of the tube at 135^{o}C using paraphine oil and expandling it by air inflation using 1 ml syringe. This cuff was placed around the common carotid artery and fixed on the sternomastoideus muscle. The rest of the tube was guided subcutaneously to the neck of the rat. Common carotid occlusion (CCO) was established by inflating the cuff and verified from the Doppler flow signal. Baroreceptor unloading was induced by CCO in conscious rats.

2.7 Plasma renin concentration measurements

The assay was performed at the department of Internal Medicine, Dijkzigt Hospital, Erasmus University, Rotterdam, The Netherlands, in cooperation with the group of F.H.M. Derks and M.A.D.H. Schalekamp.

Plasma renin concentration (PRC) was quantified by measuring the rate of angiotensin I (AI) formation by renin. Therefore under optimal conditions the unknown renin containing plasma was incubated for one hour with endogeneous plasma substrate to generate AI. However, plasma angiotensinases and converting enzyme must also be inhibited in order to protect the newly formed AI from destruction or formation to AII. The AI formed was then quantitated by radioimmunoassay.

Via a catheter in the abdominal aorta blood samples (approximately 0.5 ml) were collected into ice-chilled tubes containing 5 µl

Na_2 EDTA (100 mg/ml) and O-phenantrolin (11 mg/ml). Plasma and cells were separated by centrifugation at +4°C, 2000 rpm and plasma was stored at -80°C until assayed.

Fifty µl of plasma and 150 µl renin substrate were mixed with 100 µl phosphate buffer (pH 6.6) and 13.5 µl angiotensine and converting enzyme inhibitor solution. The renin substrate (plasma with a minimum of plasma renin activity) was prepared from rat plasma which was taken at least 24 hr after bilateral nephrectomy.

Fifty-µl portions of this mixture were incubated in triplicate at 37°C for 1 hour. One 50 µl blanc sample, wich allowed correction for the endogenous AI was taken at t=0 of the incubation time and immediately ice-cooled to stop the reaction. The phosphate buffer contained 12.2 mM NaH_2PO_4, 86.7 mM Na_2HPO_4, 75.9 mM NaCl and 1.0 mM Na_2EDTA. The inhibition solution was a mixture of a 5% (w/v) phenylmethyl sulfonyl fluoride (PMSF) solution, a 10^9 IU/ml trasylol, a 100 mmol/l Na_2 EDTA solution, and a 10% (w/v) neomycin sulphate solution in a 1:2:2:2 (v:v:v:v) ratio. After the incubation 50 µl portions were used for the radioimmunoassay to determine the formed AI. These 50 µl samples were mixed with 250 µl tris/ acetate buffer, pH 7.5, 50 µl antiserum and 50 µl ^{125}I-AI solution (±7000 cpm) and incubated overnight by 0°C.

The ^{125}I-labeled Ile^5-AI and anti-Ile^5 AI rabbit antiserum were prepared as described previously (Derks et al, 1978). The Tris acetate buffer contained 0.1 M Tris, 0.35% (w/v) bovine serum albumin, 0.1% (w/v) lysozyme, and 0.2% (w/v) neomycinsulphate. The pH was adjusted with glacial acetic acid.

The next morning 0.5 ml of a charcoal suspension was added to the incubate to separate the antibody bound AI. The charcoal suspension was prepared by mixing 2.5 g of Dextran T70 (pharmacia) and 25 g of charcoal (Sigma Chemicals MO 63178) with 1 l barbital buffer (131 mM NaCl, 7.1 mM sodium barbital and 7.1 mM sodium acetate, pH 7.5). The tubes with this mixture were shaken carefully and then centrifugated (2000 rpm, 10 min) at room temperature. The charcoal precipitate and the supernate were counted separately in a gamma counter (LKB). Calculations of plasma renin activity were described previously (Fyhrquist et al, 1976) and PRC is expressed as ng AI generated per ml

plasma per hour.

2.8 Renal hemodynamic measurements

In this thesis, two different methods were used to determine glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), measured as plasma clearances of ^{51}Cr-EDTA and ^{125}I-PAH. Both methods have been described previously by Smits et al (1983; method A) and Daemen et al (1987; method B). The later developed method B has the advantage above method A that it allows a continuous urine sampling during the experiment. During surgery rats were under ether anesthesia.

2.8.1 Method A

One day before the experiment animals were equipped with venous polyethylene (PE-10) catheters in the right femoral vein for bolus injections and an arterial catheter in the right femoral artery for blood sampling. Repeated blood sampling during 90 min after bolus injections of 10 µCi of ^{51}Cr-EDTA (CJ 13p Radiochemical Center, Amersham, England) and 10 µCi of ^{125}I-PAH (IM 315P Radiochemical Center) and subsequent fitting of obtained plasma curves to a two-compartment open model allowed simultaneous calculation of GFR and ERPF. GFR and ERPF are expressed as milliliters per minute per gram of kidney weight. Filtration fraction (FF) was calculated as GFR/ERPF.

2.8.2 Method B

Three-5 days before the experiments a special ethylene-vynil acetate copolymer dome was implanted into the bladder (the construction is described in section 2.2) allowing continuous urine sampling in conscious animals. One day before the experiment two venous catheters were implanted into the right femoral vein and one arterial catheter into the right femoral artery. Via one of the venous catheters a bolus injection of 0.5 µCi ^{51}Cr-EDTA and 1 µCi ^{125}I-hippurate (CJ, 13P respectively IM, 315P, Radio Chemical Center Amersham) was given followed by an infusion of a mixture of both tracer substances

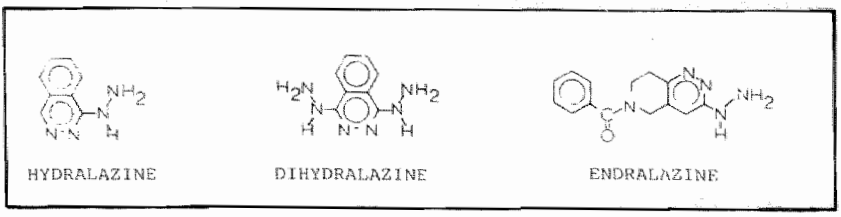
to deliver ^{51}Cr-EDTA and ^{125}I-PAH at rates of 0.6 µCi/hr and 1.8 uCi/hr respectively. After reaching a steady state for the tracers in plasma and urine, the blood (via the arterial catheter) and urine (via the bladder catheter) sampling was started. Urine samples were collected continuously in two 15-min periods before the bolus injection and in 30-min periods during the following 3 hr. Mid-times, blood samples were taken, assuming that ^{51}Cr EDTA and ^{125}PAH concentrations in these samples are representative for one whole period. GFR and ERPF were calculated from urine and plasma concentrations of both radioactive substances according to standard methods. FF was calculated as GFR/ERPF.

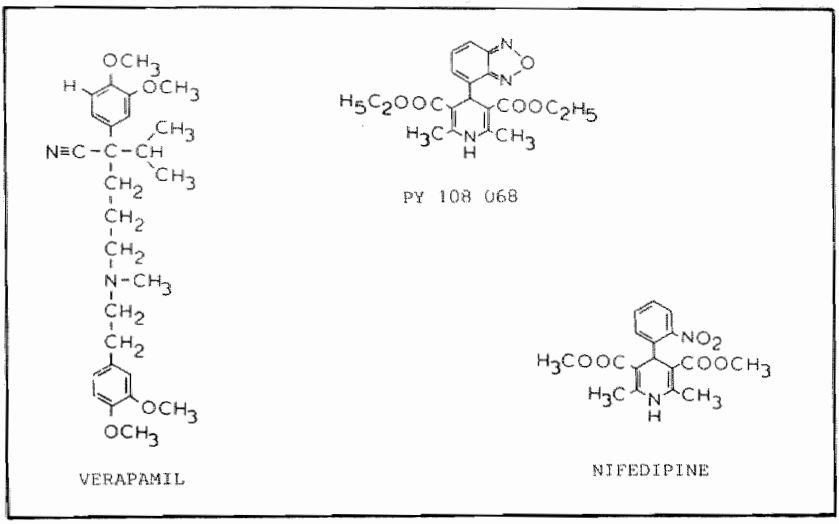
2.9 Renal excretory function measurements

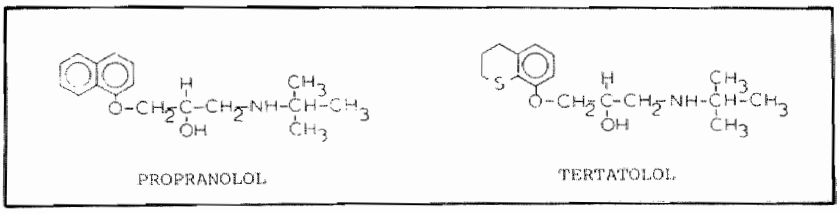
Urine and sodium excretion were quantitated in conscious SHR that were in carefully controlled balance as described in detail previously (Smits et al, 1982). Animals were implanted with catheters in the vena cava and abdominal aorta. After 2 to 3 days of recovery, during which animals had free access to standard lab food and water, they were placed in metabolic cages (Techniplast; Buguggiate Varese, Italy). Normal food was replaced by sodium-depleted food (Hope-Farms, Woerden, the Netherlands) and sodium and water intake were controlled by a continuous i.a. infusion of a Ringer's solution (9.0 g NaCl, 0.20 g KCl and 0.20 g $CaCl_2$ per liter, adjusted to pH 7.40 with a phosphate buffer) at a rate of 0.92 ml/min. The outlet of the metabolic cages was connected to an LKB model 700 fraction collector which was set to change (preweighed) tubes every 120 min. V was determined every 2 hr by weighing the tubes. Na^+-concentrations were measured using a flame photometer.

2.10 Substances used in this thesis

The chemical structure of the antihypertensive drugs used in the studies described in this thesis are presented in fig. 2.4. In chapter







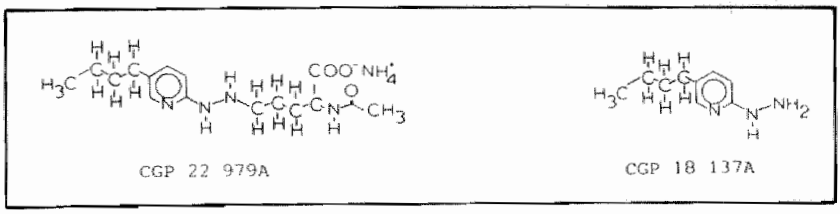


Fig. 2.4: Chemical structures of the different substances studied in chapter 4-7 of this thesis.

4, hydralazine hydrochloride (Ciba Geigy) and hydralazine-like substances as dihydralazine hydrogen sulphate (Ciba-Geigy) and endralazine (Sandoz) were investigated. All these drugs were dissolved in 0.9% NaCl (saline).

In chapter 5, the phenylalkylamine calcium entry blocker verapamil hydrochloride (Knoll) and two dihydropyridine derivates nifedipine (Bayer) and PY 108-068 (Sandoz) were used. Verapamil was dissolved in 0.9% NaCl and the two dihydropyridines in a mixture of polyethylene glycol, ethanol and 0.9% NaCl (v/v/v, 1/1/2).

In chapter 6, the non-selective beta-adrenoceptor blocking drugs propranolol (Sigma Chemicals) and tertatolol (Servier) racemates, both with no intrinsic sympathomimetic activity, were used in the described studies. These substances were dissolved in 0.9% NaCl.

In chapter 7, two experimental substances were investigated, the prodrug CGP 22 979A and its parent compound CGP 18 137A (Ciba-Geigy), both dissolved in 0.9% NaCl. CGP 22 979A has no vasodilator activity of its own but a hydrolysis by acylase and glutamyl transpeptidase is needed to generate the active hydralazine-like vasodilator CGP 18 137A.

2.11 Statistics

In the following chapters, three different statistical methods were used to compare hemodynamic values in several experimental groups:

1. Student's t-test for unpaired observations. This method was used to compare maximally two groups of unpaired values in one study.
2. One-way analysis of variance and a modified t-test for multiple comparison, following the Bonferroni method (Wallenstein, 1980). This method was used to compare more than two groups of unpaired values in one study with a one-way analysis of variance followed by a unpaired comparison with the modified t-test.
3. Analysis of variance for repeated measurements (Zerbe, 1979). This method was used to compare the values of two groups with repeated measurements.

A differentiation of these methods over the several types of experiments are presented below.

2.11.1 Baseline values

Absolute values measured before a bolus injection or start of infusion were compared with method 1. Only if more than two groups were used in one study, baseline values were compared using method 2.

2.11.2 Central hemodynamic studies

In these experiments, a comparison was made between the effects in the control group and one group receiving a particular dose of a drug. The values were measured when maximal effects were reached or at one time period. Also differences between central hemodynamics at one dose in intact and sino-aortic baroreceptor denervated animals were compared with method 1.

2.11.3 Acute regional hemodynamic studies

Similarly as in the central hemodynamic studies, the values of the control group were compared with the values in the drug treated group at one particular dose using method 1.

The regional hemodynamic dose response curves in intact and sino-aortic baroreceptor denervated animals were compared with method 3 because more than one dose was used in one group to determine these effects.

2.11.4 Renal hemodynamic and excretory function studies

In these studies, method 2 was used to compare the effects in the independent groups at one particular time period after the bolus injection. The animals received per group only one dose of one drug.

2.11.5 Long-term regional hemodynamic studies

In these studies, regional hemodynamic effects were measured every day over a period of several days. Method 3 for repeated measurements was used to compare these long-term effects between two differently treated groups. The animals received one infusion per group.

2.11.6 Plasma renin concentration studies

In these studies, plasma renin concentration was determined just before and after a 4-day infusion period of several substances. The measured absolute values were statistically compared with the control values at particular times (day 0 or day 4). When two independent groups were used in the study method 1 was used, but in the case of more than two groups, method 2 was used for comparison.

CHAPTER 3

EFFECT OF BAROREFLEX ACTIVATION ON REGIONAL HEMODYNAMICS IN CONSCIOUS NORMOTENSIVE RATS

3.1 Introduction

As described in chapter 1, the baroreflex is an important mechanism in the short-term control of the cardiovascular system. In the following chapters, the hemodynamic effects of some classes of antihypertensive drugs are described. These substances reduce blood pressure and consequently, activate sino-aortic barorecepor reflex mechanism. This leads to further changes in vascular flow and resistance counterregulating the fall in blood pressure. In those studies, the role of the baroreceptor reflex in the acute regional hemodynamic effects of the drugs were investigated in intact and sino-aortic baroreceptor denervated animals. Those studies do not answer the question whether the regional specificity in the intact animals is the result of an additive direct effect of the drug and the reflex on the vascular beds or an interference of the drug with the sino-aortic baroreflex pathway. This question may be answered if the regional hemodynamic effects of a baroreceptor reflex activation are known. Therefore, the study described in this chapter was designed to determine regional hemodynamic effects, induced by a non-pharmacological baroreflex activation.

Some investigators have used non-pharmacological methods to activate the baroreflex mechanism to investigate regional hemodynamics. Bond and Green (1969) induced a carotid sinus pressure reduction by bilateral carotid occlusion in anesthetized dogs and observed a baroreflex-mediated increase in vascular resistance in the viscera, kidney and skeletal muscle. Cox and Bagshaw (1980) activated the baroreflex by controlled external saline perfusion of the carotid sinus area and found a similar inverse relationship between carotid

sinus pressure and resistance in mesenteric, renal and femoral vascular beds. In these experiments, it cannot be excluded that the anesthesia (Cox and Bagshaw, 1979) or the strongly reduced cerebral blood flow influences the baroreflex-induced hemodynamic effects. Therefore, in this study we have activated the baroreflex in conscious WKY rats by unilateral common carotid occlusion (CCO) and investigated its effect on regional vascular resistance. The baroreceptors on the aortic arch and other bifurcation were denervated to avoid a counter-regulation by these baroreceptors. In separate studies we determined the relationship between carotid sinus pressure and systemic MAP during CCO in anesthetized rats and the direct effect of CCO on MAP and HR in conscious totally denervated rats. In addition, we have checked the influence of such partial denervation on baroreflex sensitivity.

3.2 Experimental protocols

3.2.1 Animals

Male normotensive Wistar-Kyoto (WKY) rats of age ranging from 3-4 months were used in all the studies described in this chapter (for more details, see paragraph 2.1).

3.2.2 Protocol for denervation and baroreflex sensitivity measurements

4 Days before the experiments 5 groups of WKY rats were subjected to either total, unilateral or sham denervation. The baroreceptor denervation is described in section 2.5. In the unilaterally denervated group the innervation of the baroreceptors on the right carotid sinus was left intact. Three days later, under ether anesthesia, a catheter was placed into the abdominal aorta for MAP and heart period (HP= 60.000/HR) measurements. Bolus injections of phenylephrine were administered via a catheter in the vena cava. On the experimental day, baroreflex sensitivity (BRS) was determined in the 3 different groups by linear regression analysis of HP and MAP induced by different doses of phenylephrine. The slope was used as an index for BRS, if $p<0.05$.

3.2.3 Protocol for determining the relationship between carotid sinus pressure (CSP) and systemic MAP

Surgery and measurements were performed in pentobarbital anesthetized animals. On the experimental day an occluder was placed around the left common carotid artery and the superior thyroid artery was cannulated in a retrograde fashion with the tip in the carotid sinus. The femoral artery was cannulated for MAP measurements and the femoral vein for infusions. MAP was varied by infusions of angiotensin II and nitroprusside sodium. At several levels of MAP, a CCO was performed. The relationship between MAP and CSP was determined by linear regression analysis.

3.2.4 Protocol for carotid occlusion and measurement of regional hemodynamics

Regional blood flows were measured using a 20 MHz directional pulsed Doppler system with miniaturized Doppler flow probes according to Haywood et al (1981).

Six days before the experiments the rats were unilaterally denervated as described in chapter 2. In addition, an occluder and Doppler flow probe were placed around the right (innervated) common carotid artery. Three days later, Doppler flow probes were implanted around the renal, superior mesenteric artery and abdominal aorta. After 2 days of recovery the abdominal aorta was cannulated for MAP and HR measurements. On the experimental day the animals were connected to the registration equipment. They were allowed to habituate to the experimental conditions for at least 1 hr. Thereafter, the changes in MAP, HR, renal (RF), mesenteric (MF) and hindquarter (HQF) flow to steady-state levels, induced by a 20-s unilateral CCO period were determined and resistance changes were calculated. In a separate group of totally denervated animals, the effect of CCO on MAP and HR was measured.

3.3 Results

3.3.1 Effect of denervation on BRS

Pre-injection values of MAP and HR in the sham-operated, in the

*Table 3.1: Pre-injection values of MAP(mm Hg) and HR(bpm) in the sham-operated unilaterally denervated and totally denervated group (mean ± SEM). Significant differences are given in comparison to sham denervation: *p<0.001.*

Denervation	n	MAP	HR
Sham	8	128±3	341±7
Unilaterally	8	141±6	387±10*
Totally	7	136±6	393±11*

unilaterally denervated, and in the totally denervated group, are summarized in table 3.1. In unilaterally and totally denervated animals, HR pre-injection values were significantly higher ($p<0.001$) than in sham-operated rats.

Fig. 3.1 shows typical regression lines for HP vs. MAP following intravenous phenylephrine in sham, unilaterally and totally denervated rats. The slope was used as an index for baroreflex sensitivity. BRS was 1.5± 0.8 ms/mm Hg (n=8) in intact animals. After unilateral denervation, BRS was significantly lower (0.6±0.3 ms/mm Hg; n=8; $p<0.01$). Total baroreceptor denervation virtually abolished BRS (0.14±0.9 ms/mm Hg; n=7; $p<0.02$).

3.3.2 Relationship between systemic MAP and CSP during CCO

Before occlusion, CSP equalled MAP. The relationship between MAP and CSP during CCO may be described as CSP = 0.61xMAP + 3.1 mm Hg with a coefficient of correlation of 0.93 (fig. 3.2).

3.3.3 Effect of unilateral CCO on regional hemodynamics

Pre-occlusion values of MAP and HR were 134±17 mm Hg and 406±18 bpm in the totally denervated, and 109±7 mm Hg and 357±14 bpm in the unilaterally denervated group.

The hemodynamic changes induced by unilateral CCO are summarized in fig. 3.3.

In totally denervated rats, CCO did hardly affect MAP (2±1%) and HR (2±1%). In unilaterally denervated animals, MAP increased (28±4%). This was associated with vasoconstriction in all 3 vascular beds (RR: 33±8%; HQR: 27±7%, and MR: 24±5%), but not by flow changes (RF: -3±1%; HQF: -0.6±3%, and MF: 4±3%). This vasoconstriction was more pronounced in the kidney than in hindquarters and mesentery.

3.4 Discussion

In this study, we have investigated a non-pharmacological baroreflex activation by unilateral CCO in conscious rats. The results

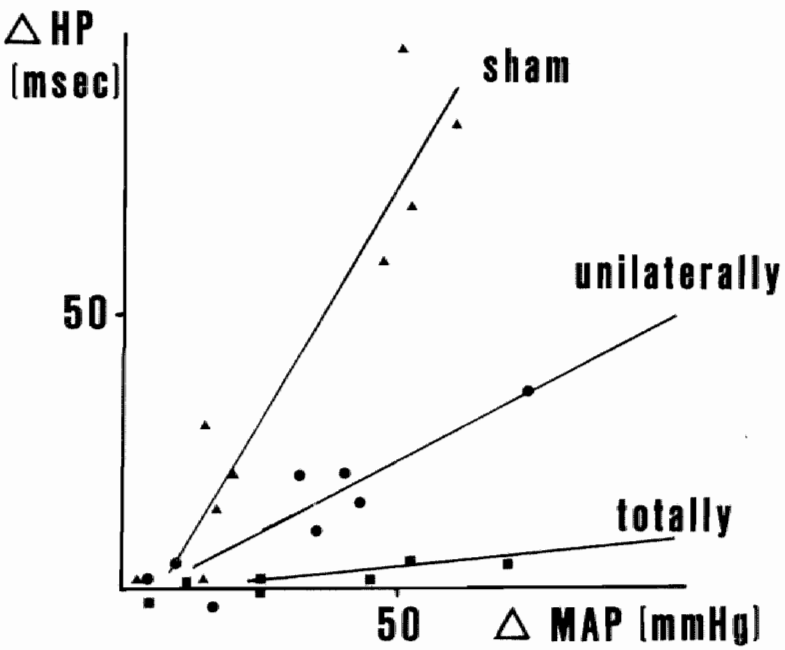


Fig. 3.1: Typical regression lines for mean arterial pressure (MAP) vs. heart period (HP) following intravenous phenylephrine in sham, unilaterally and totally denervated rats (y=1.6x-13; r=0.89; y=0.5x-3.2; r=0.91; y=0.1x-1.4; r=0.89 respectively).

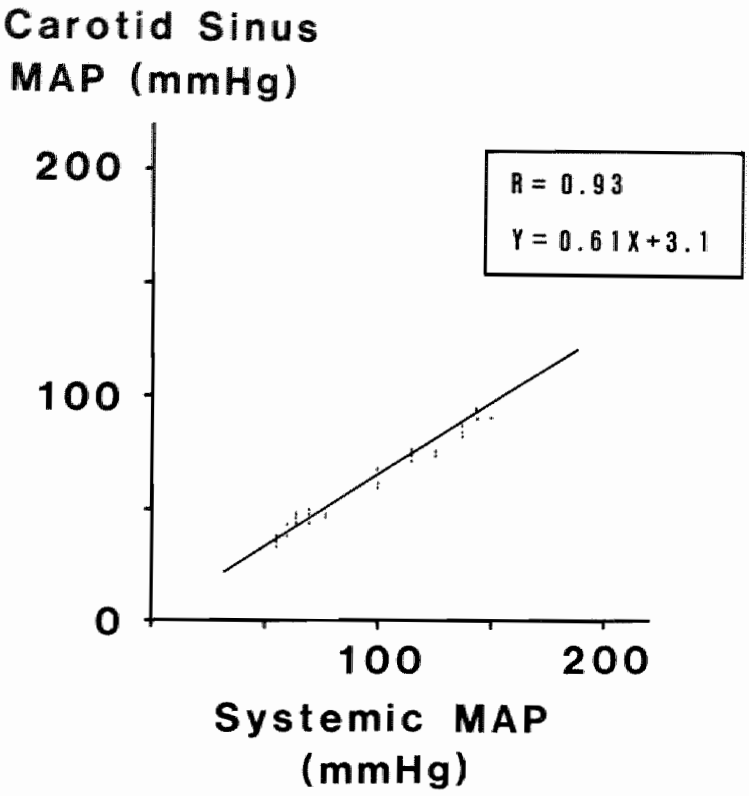


Fig. 3.2: Correlation between the carotid sinus pressure and systemic MAP during carotid occlusion.

show that unilateral CCO reduces CSP by approximately 40% (fig. 3.2). Via the baroreceptors located in this carotid sinus area the pressure reduction activates the baroreflex mechanism, inducing hemodynamic changes. The baroreceptors on the aortic arch and other bifurcation were denervated to avoid counter-regulation by these baroreceptors. It was therefore necessary to test first in a separate study how unilateral baroreceptor denervation affects the baroreflex. The results indicate that unilateral denervation reduces BRS but does not abolish the baroreceptor reflex (fig. 3.1). Furthermore, unilateral CCO did not affect systemic MAP in totally denervated animals. We therefore conclude that CCO can be used as a non-pharmacological method to

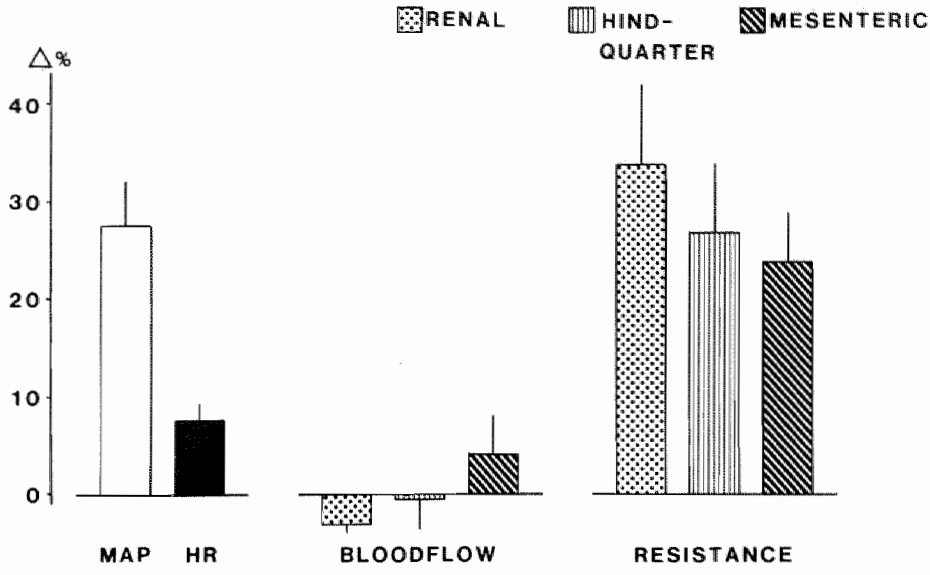


Fig. 3.3: Effects of baroreflex activation induced by unilateral carotid occlusion on mean arterial pressure (MAP), heart rate (HR) and flow and resistance in the renal, hindquarter and mesenteric vascular bed.

activate baroreflex mechanism in conscious small animals.

The second purpose of this study was to investigate the regional vascular effects of baroreflex activation induced by CCO. The implanted miniaturized Doppler flow probes allowed simultaneous measurement of flow changes in different vascular beds (Haywood et al, 1981). The results indicate that the baroreflex mechanism activated by unilateral sinus pressure reduction, increases MAP by a vasoconstriction in the renal, hindquarter and mesenteric vascular bed, which is most pronounced in the kidney (fig. 3.3). Bond and Green (1969) have shown that activation of the baroreflex mechanism by bilateral carotid occlusion increases vascular resistance in kidney, skeletal muscle and viscera in anesthetized dogs to a similar degree. Cox and Bagshaw (1980), using a controlled external saline perfusion of the carotid sinus area to activate the baroreflex mechanism in anesthetized dogs, found general vasoconstriction which was most pronounced in the femo-

ral and least pronounced in the mesenteric vascular bed. These results indicate that carotid sinus hypotension induces a baroreflex mediated general vasoconstriction with some contrary regional specificity in the different studies. It is not clear whether these differences derive from species difference or alternatively from the lack of anesthetic use in the present study.

CCO did hardly change HR and flow through the three vascular beds. A much smaller effect on cardiac output than on total peripheral resistance was also observed by Kirchheim and Gross (1971) during bilateral CCO in conscious dogs. A possible explanation for these observations could be that baroreceptors on the aortic arch are responsible for the baroreflex mediated effects on HR and cardiac output because baroreceptor unloading with depressor substances like nitroprusside sodium which also unloads baroreceptors on the aortic arch causes strong HR increases (Harron et al, 1984).

Green and Rapela (1965) suggested that autoregulation of the vascular beds increases vascular resistance during CCO. This hypothesis could explain the lack in flow changes during CCO in the present study. This was supported for the kidney by Higgins et al (1973), Kirchheim (1969) and Kirchheim and Gross (1970) using alpha-adrenoceptorblocking agents to block the sympathetic vascular responses. In similar studies, Bond and Green (1969) showed that the increase in mesenteric resistance could at least in part be due to autoregulation. The resistance increase in skeletal muscle during CCO was totally abolished after alpha-blockade or after removal of the abdominal sympathetic chain (Folkow and Neil, 1971), suggesting an increased sympathetic tone in the muscular vascular bed during CCO.

In summary, the results show that baroreceptor activation leads to a vasoconstriction in all vascular beds studied. Possibly, the increase in vascular resistance is mediated by different mechanisms, e.g. autoregulation and increased sympathetic activity. This was not further investigated in the present study.

CHAPTER 4

HEMODYNAMIC EFFECTS OF HYDRALAZINE AND SOME HYDRALAZINE-LIKE ARTERIOLAR VASODILATORS IN THE CONSCIOUS SPONTANEOUSLY HYPERTENSIVE RAT

4.1 Introduction

Hydralazine and hydralazine-like vasodilators are given as a third step after beta-adrenoceptor blockers and/or diuretics in the treatment of hypertension. This is the result of several less favorable effects occurring during monotherapy with these drugs (Koch-Weser, 1974; Vidrio and Tena, 1980). The acute hemodynamic effects of arteriolar vasodilators have been described extensively. In hypertensive man, such studies are difficult to interpret because of the usually combined use of these vasodilators and beta-adrenoceptor blocking drugs (Zacest et al, 1972; Koch-Weser, 1974; Brunner et al, 1978). In animal studies, an interference of beta-adrenoceptor antagonists with the hemodynamic effects of vasodilators is suggested by Gutkin et al (1977) and Bolt and Saxena (1984a). Therefore, the first study in this chapter was designed to determine the central hemodynamic effects of the vasodilators hydralazine, dihydralazine and endralazine in conscious SHR. We used a technique for measuring continuously cardiac output from an implanted electromagnetic flow probe on the ascending aorta as described originally by Smith and Hutchins (1979) and developed further by Smits et al (1982).

The primary pharmacological effect of these agents is a relaxation of vascular smooth muscles which leads to a (rapid and relatively strong) fall in total peripheral resistance and blood pressure. Relatively little is known about which vascular beds are responsible for the strong fall in total peripheral resistance. The few available regional hemodynamic studies in man (Zins, 1974; Leier et al, 1981) and dogs (Chelly et al, 1986) indicate that hydralazine is a renal vasodilator. However, Bolt and Saxena (1984a) observed not only a

renal vasodilation, but also a vasodilation in heart and brain and even an increase in vascular resistance in skin and gastrointestinal tract after hydralazine.

In a second study we have investigated the regional hemodynamic effects of hydralazine in conscious SHR, using a 20-MHz directional pulsed Doppler system according to Haywood et al (1981).

The strong blood pressure reduction after directly acting arteriolar vasodilators causes a reflex increase in sympathetic nerve activity to the heart and the blood vessels changing the direct hemodynamic effects of these drugs. In a final study, we assessed the role of the baroreceptor mediated reflex mechanisms in the hemodynamic effects of hydralazine. Therefore, acute central and regional hemodynamic effects of this drug were determined in sino-aortic baroreceptor denervated and non-denervated SHR.

4.2 Experimental protocol

Surgery, measurements and calculations concerning the central and regional hemodynamics were performed as described in section 2.2 and 2.4. of this thesis. The baroreceptor denervation is described in section 2.5. On the experimental day, the animals were placed in experimental cages between 9.00 and 10.00 a.m. After the catheters and flowprobe cables had been connected to the registration equipment, the animals were allowed to habituate to the experimental conditions for 1 hr. Control values (t=0 in the results) were obtained as the average of 4 readings at 5-min intervals in the last 20 min before an injection.

To determine the central hemodynamic effects, 5 different experimental groups received intravenously (i.v.) 0.1 ml 0.9% NaCl solution (saline), 0.3 mg/kg hydralazine hydrochloride (Ciba-Geigy), 0.3 mg/kg dihydralazine hydrogen sulphate (Ciba-Geigy), and 0.1 and 0.3 mg/kg endralazine mesilate (Sandoz), respectively. In another group of sino-aortic baroreceptor denervated SHR the central hemodynamic effects of 0.3 mg/kg hydralazine were measured.

The regional hemodynamic effects of 0.1-1.0 mg/kg hydralazine

Table 4.1: Control values (± SD) for central hemodynamic variables in the different experimental groups. Units: mean arterial pressure (MAP), mm Hg; cardiac index (CI), ml/min.100 g bw; total peripheral resistance index (TPRI), mm Hg.100 g bw/ml; heart rate (HR), bpm; stroke volume index (SVI), µl/100 g bw.

		n	MAP	CI	TPRI	HR	SVI
Saline		9	145±17	35.4±5.2	4.0±0.3	398±18	92±20
Hydralazine	0.3 mg/kg	8	156±9	33.9±2.4	4.8±0.4	402±35	84±13
Dihydralazine	0.3 mg/kg	7	144±11	31.1±9.8	5.1±1.7	376±45	83±25
Endralazine	0.1 mg/kg	5	159±13	31.9±5.3	5.1±1.1	393±43	81± 9
Endralazine	0.3 mg/kg	7	158±13	34.6±4.5	4.7±0.9	405±33	87±11

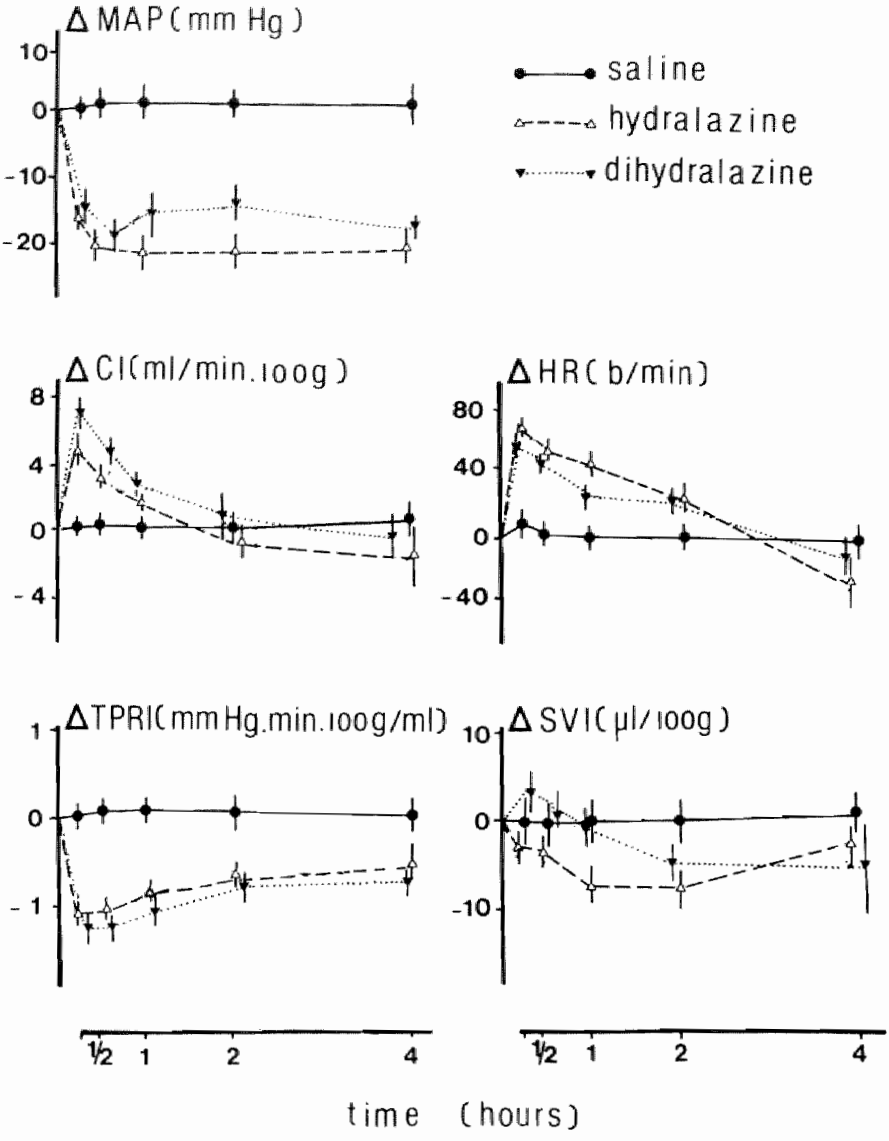


Fig. 4.1: Effects of 0.1 ml saline, 0.3 mg/kg hydralazine and 0.3 mg/kg dihydralazine on mean arterial pressure (MAP), cardiac index (CI), total peripheral resistance index (TPRI), heart rate (HR), and stroke volume index (SVI) in conscious SHR.

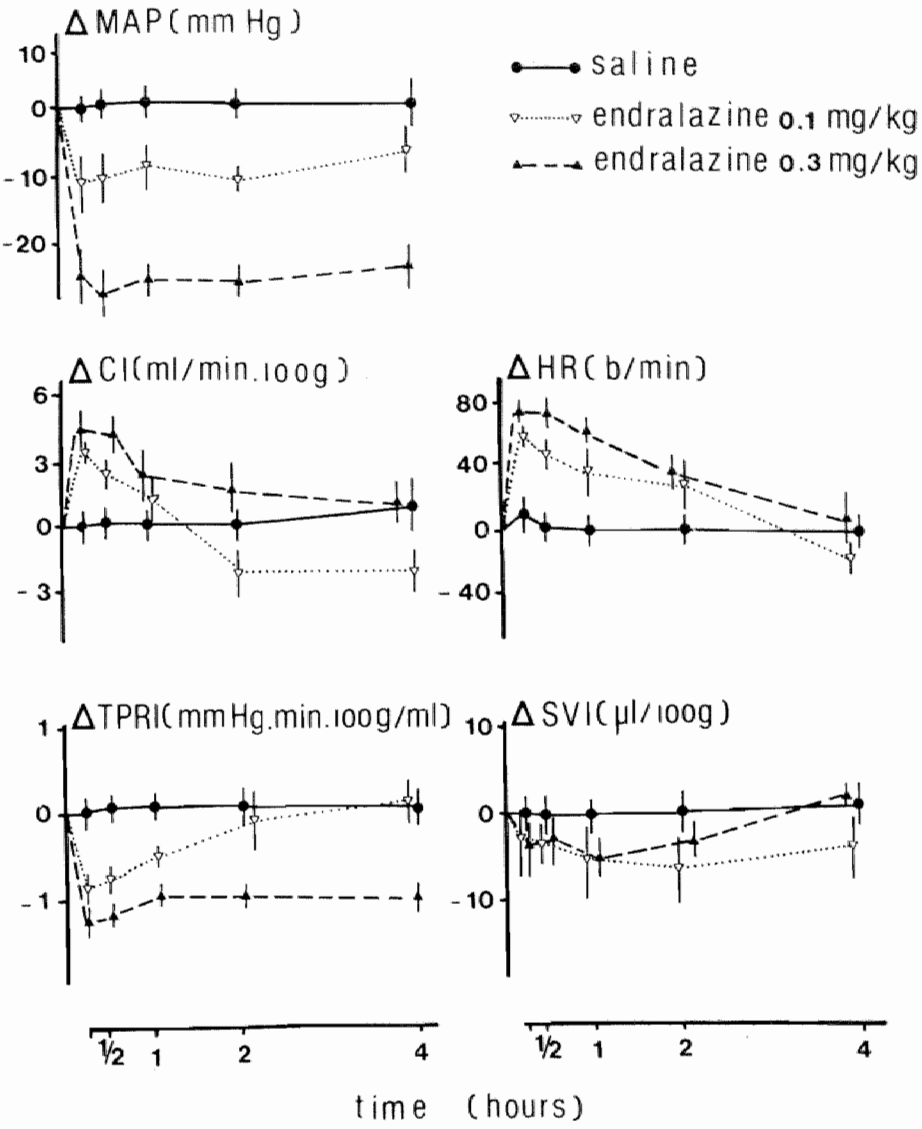


Fig. 4.2: Effects of 0.1 ml saline, 0.1 mg/kg endralazine and 0.3 mg/kg endralazine on mean arterial pressure (MAP), cardiac index (CI), total peripheral resistance index (TPRI), heart rate (HR), and stroke volume index (SVI) in conscious SHR.

were determined in baroreceptor-denervated and non-denervated SHR.

4.3 Results

4.3.1 Central hemodynamic effects of hydralazine and dihydralazine

Control values for the different groups of rats are summarized in table 4.1. There were no statistically significant differences between the control values of the hemodynamic variables for the different groups.

In pilot experiments with separate animals, doses of the 2 vasodilators were chosen that would cause a comparable fall in MAP. The hemodynamic studies were thus performed with 0.3 mg/kg hydralazine (n=8) and 0.3 mg/kg dihydralazine (n=7), whereas the control group (n=9) received 0.1 ml 0.9% NaCl. Both vasodilators caused a rapid fall in MAP (fig. 4.1). A maximum decrease was reached after 1 hr, amounting to -21 ± 3 mm Hg ($p<0.001$) for hydralazine and -18 ± 3 mm Hg ($p<0.001$) for dihydralazine. MAP then slowly returned towards its control values. After 24 hr, MAP in the hydralazine group did not differ significantly from that in the saline treated rats. Dihydralazine at that time still caused a significant ($p<0.001$) fall of -12 ± 2 mm Hg compared to $+2\pm4$ mm Hg in the saline group. The fall in MAP was paralleled by a decrease in TPRI (fig. 4.1). A maximal decrease of -1.1 ± 0.1 ($p<0.001$) and -1.3 ± 0.3 ($p<0.001$) mm Hg.min.100 g bw/ml was observed for hydralazine and dihydralazine respectively within 30 min after injection. In the hydralazine treated group, TPRI returned to control levels at 24 hr whereas a significant ($p<0.05$) decrease of -0.8 ± 0.3 (vs. $+0.1\pm0.3$ in the saline group) mm Hg.min.100 g bw/ml was still observed for the dihydralazine group.

Both vasodilators caused a rapid increase in CI and HR (fig. 4.1). The maximum for this increase was observed within 30 min. For CI, the rise amounted to 4.7 ± 0.7 ($p<0.001$) and 6.5 ± 1.3 ($p<0.001$) ml/min.100 g body weight for hydralazine and dihydralazine, respectively. The maximal increases in HR were 66 ± 8 bpm ($p<0.001$) and 60 ± 9 bpm ($p<0.001$) for hydralazine and dihydralazine respectively. In contrast to the long-lasting decreases of MAP and TPRI, CI and HR re-

turned to their control values within 1-2 hr. After this period, no significant differences were observed between the experimental and control groups with respect to CI and HR. No significant changes were observed in SVI, although there was a tendency to a decrease after hydralazine.

4.3.2 Central hemodynamic effects of endralazine

The hemodynamic effects of a range of doses (0.1-1 mg/kg) of endralazine were studied. Fig. 4.2 summarizes the effects of 0.1 mg/kg (n=5) and 0.3 mg/kg (n=7) endralazine. One mg/kg (n=4) caused such a profound fall in MAP (-87±6 mm Hg at t=30 min) that further hemodynamic data are not included. The 2 lower doses caused a rapid fall in MAP, amounting to -11±4 ($p<0.05$; 0.1 mg/kg) and -29±5 mm Hg ($p<0.001$; 0.3 mg/kg) within 30 min. Thereafter, MAP gradually increased again although it was still reduced by 22±3 mm Hg ($p<0.001$) at 24 hr after 0.3 mg/kg. The fall in TPRI was parallel to the fall in MAP, with maximum decreases of 0.8±0.2 ($p<0.001$; 0.1 mg/kg) and 1.2±0.1 ($p<0.001$; 0.3 mg/kg) mm Hg.min.100 g bw/ ml. After 24 hr, TPRI was still reduced by 1.3±0.4 ($p<0.01$; 0.3 mg/kg) vs. an increase of 0.1±0.3 (saline) mm Hg.min.100 g bw/ ml.

Endralazine caused an immediate increase in CI and HR (fig. 4.2) with a maximum reached within 30 min. The increase in CI amounted to 3.7±0.4 ($p<0.001$; 0.1 mg/kg) and 4.5±1.1 ($p<0.001$; 0.3 mg/kg) ml/100 g bw. HR increased by 57±15 ($p<0.001$; 0.1 mg/kg) and 70±15 ($p<0.001$; 0.3 mg/kg) beats/min. CI returned to control levels within 1 hr, whereas HR was back after 2 hr. No significant changes were observed with respect to SVI.

4.3.3 Influence of baroreflex denervation on the central hemodynamic effects of hydralazine

Table 4.2 summarizes the baseline values for hemodynamic variables in the non-denervated SHR (n=8) and the baroreflex-denervated animals (n=7). MAP was significantly ($p<0.05$) higher in the denervated SHR, due to a large increase ($p<0.001$) in TPRI. HR was not significantly different, but CI and SVI were both significantly ($p<0.001$) lower in the denervated SHR. The maximal effects of 0.3 mg/kg hydralazine 15-30 min after injection are given in fig. 4.3. Because of the

*Table 4.2: Baseline values (± SEM) for hemodynamic variables in the non-denervated and baroreflex-denervated SHR. Units: see table 4.1. Significance of the difference between the non-denervated and denervated animals: *p<0.05; **p<0.001.*

	Non-denervated (n=8)	Denervated (n=7)
MAP	156 ±3	174 ±7*
CI	33.9±0.8	23.8±1.2**
TPRI	4.8±0.2	7.4±0.3**
HR	402 ±12	428 ±15
SVI	84 ±5	56 ±3**

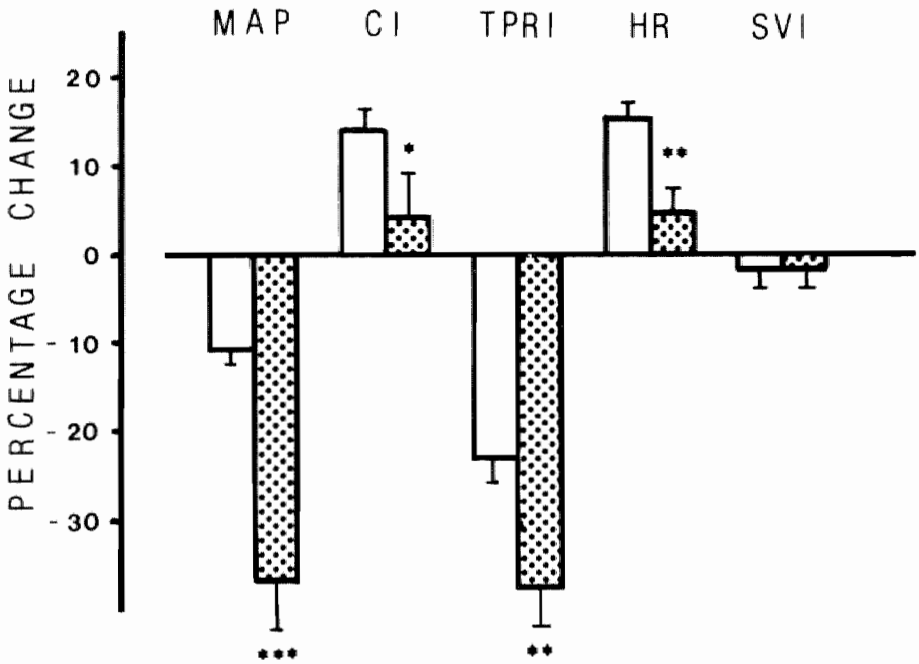


Fig. 4.3: Comparison of the maximal effects of 0.3 mg/kg hydralazine in baroreceptor-denervated SHR (dotted bars) and in non-denervated SHR (open bars) on mean arterial pressure (MAP), cardiac index (CI), total peripheral resistance index (TPRI), heart rate (HR), and stroke volume index (SVI).

different baseline values, the effects are expressed as percentage changes. The fall in MAP was significantly ($p<0.001$) larger than in non-denervated SHR (-37±6 vs. -11±1%).

Similarly, the decrease in TPRI was larger in the denervated animals (-38±4 vs. -23±3%; $p<0.01$). The early increase in CI was almost absent in the denervated hydralazine injected animals (4±6 vs. 14±2%; $p< 0.05$). Tachycardia also was very slight in the denervated SHR (5±3 vs. 16±2%; $p<0.01$). There were no significant changes in SVI in either group.

4.3.4 Regional hemodynamic effects of hydralazine before and after baroreceptor denervation

Pre-injection values for MAP and HR in the different experimental groups used for the regional hemodynamics are summarized in table 4.3.

There were no statistically significant differences in control values of MAP and HR between the saline and hydralazine-treated groups. In contrast to what we observed in the central hemodynamic study, the baseline value of MAP did not differ significantly in the two groups. In contrast, however, HR was significantly higher in baroreceptor denervated animals than in intact animals.

The effects of hydralazine on regional hemodynamics in intact and denervated SHR are shown in fig. 4.4. Hydralazine caused a dose-dependent fall in MAP and an increase in HR with magnitudes and durations similar to those in the central hemodynamic experiments. Hind-

*Table 4.3: Pre-injection values for MAP(mm Hg) and HR(bpm) in the different experimental groups. Significance of the difference between non-denervated and denervated animals: *$p<0.05$.*

	Non-denervated			Denervated		
	n	MAP	HR	n	MAP	HR
0.9% NaCl	14	150±14	327±26	10	143±10	354±12*
Hydralazine	11	145±14	325±36	10	155±24	391±18*

quarter and mesenteric blood flow increased dose-dependently by respectively 48±17% and 22±14%. Renal blood flow only slightly increased after 0.3 mg/kg hydralazine (+14±6%). The blood pressure reduction was parallelled by a decrease in renal, hindquarter and mesenteric resistance (-24±8, -48±5, and -36±7% respectively after 1 mg/kg hydralazine). The reduction in HQR was significantly greater than the fall in renal and mesenteric resistance after 1 mg/kg hydralazine ($p<0.05$). In denervated animals the fall in MAP following hydralazine was significantly ($p<0.001$) greater than in non-denervated SHR (maximally 51±4% vs. 25±3%). The same was observed for the percentage reduction in renal ($p<0.05$), hindquarter ($p<0.05$) and mesenteric ($p<0.001$) resistance. The tachycardia after hydralazine was not observed in the denervated SHR ($p<0.001$).

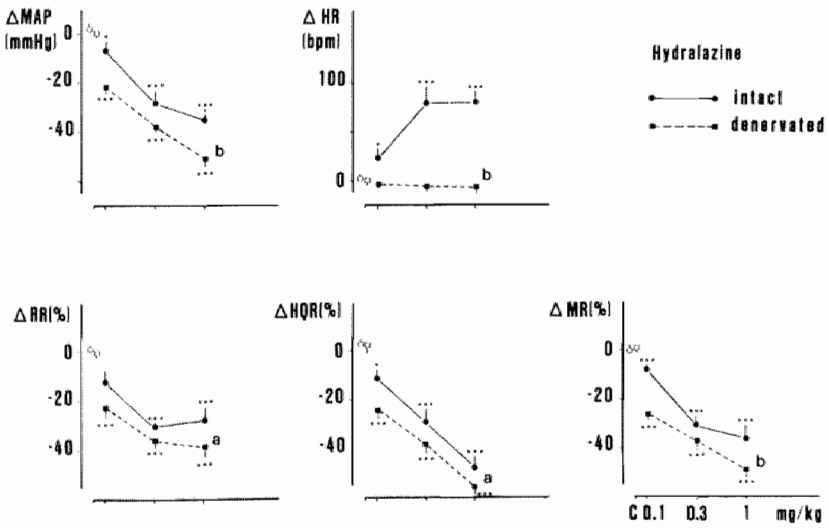


*Fig. 4.4: Effect of various doses of hydralazine on mean arterial pressure (MAP), heart rate (HR), renal resistance (RR), hindquarter resistance (HQR) and mesenteric resistance (MR) in conscious intact and sino-aortic denervated SHR. Significances are given in comparison with control injections (***$p<0.001$), and in comparison with the values in the denervated animals (a=$p<0.05$; b=$p<0.01$).*

4.4 Discussion

The animal models used in this study allow a continuous measurement of central and regional hemodynamic variables in unrestrained, conscious SHR. When studying hemodynamic effects induced by antihypertensive drugs, it is important to follow the changes with respect to time, preferably on a continuous basis. Moreover, in order to properly study the influence of nervous reflex mechanisms on drug-induced effects, the use of unanesthetized, undisturbed animals is essential.

The present data confirm the long-standing conclusion on the primary hemodynamic effects of arteriolar vasodilators, like hydralazine and dihydralazine (cf. Gross, 1977, for a detailed discussion). These agents cause an immediate fall in blood pressure with an underlying fall in total peripheral resistance. In this respect, these drugs behave differently from beta-adrenoceptor blockers or diuretics, the antihypertensive activity of which in the conscious SHR is associated with a fall in cardiac output, and even an increase in peripheral resistance (Smits et al, 1982; Struyker Boudier et al, 1983b). In this study we furthermore compared the hemodynamic effects of the new vasodilator endralazine to the activity of the arteriolar vasodilators hydralazine and dihydralazine. Some early results on the experimental and clinical pharmacology of endralazine were published previously (Salzman et al, 1979; Kirch and Exthelm, 1982; Elliott et al, 1982). On the basis of these studies, endralazine was characterized as an arteriolar vasodilator. Our studies confirm these results. Endralazine is slightly more active than hydralazine. Its duration of action is somewhat longer than that of hydralazine, an observation in agreement with clinical data (Elliott et al, 1982).

The studies with implanted Doppler flowprobes in conscious SHR indicate that the fall in total peripheral resistance after hydralazine is induced by a generalized reduction of resistance in the renal, hindquarter and mesenteric vascular bed with a most pronounced effect in the skeletal muscle. The same regional hemodynamic pattern for hydralazine was observed by Maekawa et al (1984) in dogs using the microsphere technique. Recently, Chelly et al (1986) using Doppler flow probes in conscious dogs showed that hydralazine decreases coro-

nary and renal resistances. Furthermore, Bolt and Saxena (1984a) observed a vasodilation in the heart, brain, kidneys and large intestine but a vasoconstriction in skin, stomach and small intestine using the radioactive microsphere technique in hypertensive rabbits. Only few systematic attempts have so far been made to distinguish between the sensitivity of arteries of different vascular beds.

All vasodilators used in this study caused an increase in cardiac output and heart rate. We could not confirm an earlier conclusion (Salzman et al 1979) that the reflex increase in cardiac output and heart rate for a given fall in blood pressure is less after endralazine than after hydralazine. These effects have been related to a direct cardiac action of the vasodilators (Khatri et al, 1977; Wendling et al, 1979) or to a reflex-mediated increase in sympathetic activity (Vidrio and Tena, 1980; Pérez et al, 1982). The present study clearly supports the concept of a baroreflex mediated increase in sympathetic nerve activity. Any given dose of hydralazine caused a significantly larger fall in blood pressure and peripheral resistance in the SHR without baroreflex control than in the non-denervated animals. Significantly larger falls in regional resistances after denervation were found in all 3 vascular beds studied. These results indicate that the general vasodilation seen in intact animals after hydralazine is an additive effect of a strong direct vasodilation by hydralazine and a vasoconstriction induced by baroreceptor reflex desactivation (see chapter 3). The remaining small increase in cardiac output and heart rate in the central hemodynamic groups might have been caused either by a slight direct positive chronotropic effect of hydralazine (Pérez et al, 1982).

In the central hemodynamic studies, surgical afferent denervation of the baroreceptor reflex led to a slight further increase in blood pressure in the SHR, in combination with an increase in total peripheral resistance. This observation agrees with earlier results in baroreceptor-denervated normotensive rats (Krieger, 1967), rabbits (Alexander and DeQuattro, 1974), and foxhounds in which the baroreflex was deafferentated by lesions of the nucleus tractus solitarii (NTS) in the brain stem (Carey et al, 1979). Cardiac output and stroke volume were even significantly reduced after baroreflex denervation. A

similar observation was made by Carey et al (1979) in the NTS-lesioned foxhound. These reductions in cardiac output and stroke volume are probably related to an increased afterload (Braunwald et al, 1967).

In the regional hemodynamic studies, the pre-injection values show that sino-aortic denervation does not lead to a further elevation in blood pressure in conscious SHR. Blood pressure was much more labile in these animals and heart rate was significantly higher. This effect of denervation was also observed in the other regional hemodynamic studies described in this thesis, indicating that the animal preparation might be involved. Possibly, the baroreceptors on the aortic arch are better denervated in the central hemodynamic studies as a consequence of the electromagnetic flow probe implantation on the ascending aortic arch where baroreceptors are located.

The reflex increases in cardiac output and heart rate after the administration of the arteriolar vasodilators were of short duration in comparison to the prolonged decrease in blood pressure. Whereas the fall in blood pressure lasted for up to 24 hr, reflex increases in cardiac output and heart rate were observed only for 1 to 2 hr at most. These data point to a rapid disappearance of the baroreflex-mediated increased sympathetic tone. This rapid waning of baroreceptor influences could be related to the adaptation of the baroreceptor reflex. The effects on CI, HR and SVI measured in sino-aortic baroreceptor denervated animals were almost comparabele with the effects measured 2 hr after hydralazine in intact animals. These results support the hypothesis of a rapid baroreflex adaptation. However, a significant stronger reduction in TPRI was observed in denervated as compared to intact animals. So we would expect that TPRI further decreased during the 2 hr after the bolus injection of hydralazine. This was not observed in our central hemodynamic studies. These results indicate a different baroreflex resetting time for the heart and the blood vessels induced by hydralazine. A rapid adaptation has been reported to occur within several hours in the conscious rat (Salgado and Krieger, 1978). It has previously been shown that rapid baroreflex adaptation is also involved in the early hemodynamic changes after propranolol in the conscious SHR (Struyker Boudier et al, 1979; Smits et al, 1980b, 1982). If the baroreflex adapts to a vasodilator-induced

fall in blood pressure, this might have important clinical consequences for chronic use of vasodilators. In hypertensive patients, it is also possible that the early increases in cardiac output and heart rate might disappear eventually during the long-term use of such agents. Indirect evidence for such a phenomenon in hypertensive patients was provided by Brunner et al (1978) who showed a reduced need for beta-adrenoceptor blockade in patients who were treated for several months with the arteriolar vasodilator minoxidil. A major problem in the hemodynamic analysis of such long-term effects of vasodilators is that these drugs are always administered in combination with other antihypertensives. It could therefore be of relevance to repeat our studies under conditions of long-term administration of vasodilators in conscious SHR. Long-term regional hemodynamic effects of a hydralazine-like substance, CGP 18 137A, are described in chapter 6 of this thesis.

In addition to the baroreflex-mediated changes in autonomic nerve activity, a change in plasma renin concentration (PRC) may affect the hemodynamic responses to arteriolar vasodilators. Thus, arteriolar vasodilators increase PRC, either through reflex activation of the sympathetic nervous system or through a direct renal effect of the reduced arterial pressure (Koch-Weser, 1974; Gross, 1977). It cannot be excluded that the larger fall in MAP and TPRI which we observed in baroreflex-denervated SHR as compared to the fall in intact rats after hydralazine was partly related to a smaller degree of reflex activation of PRC.

It is still not clear by what mechanism hydralazine and related substances reduce peripheral resistance and whether this mechanism is similar in all vascular beds. Some investigators suggested a direct effect on arteriolar smooth muscle (Gross et al, 1950; Khayal et al, 1981). Indirect mechanisms have also received some attention, e.g. interference with sympathetic innervation of arteriolar smooth muscle (Worcel et al, 1980) or enhanced release of endogenous vasodilator prostaglandins (Haeusler and Gerold, 1979). The data of Chelly et al (1986) who used a cyclo-oxygenase inhibitor in combination with flow measurements, indicate that the renal vasodilation after hydralazine is dependent upon the prostaglandin system. Furthermore, they did not

find a hydralazine-induced coronary vasodilation after blocking the baroreflex pathway, suggesting that the coronary vasodilation is related to an increase in myocardial oxygen demand. Meakawa et al (1984) observed that prostaglandins have their major effect on the splanchnic and renal circulation. Pre-treatment with a cyclo-oxygenase inhibitor led to an increase in renal and mesenteric resistance after hydralazine. These data may indicate that the vascular effects of hydralazine are partly mediated by effects on the prostaglandin synthesis. Meakawa et al (1984) suggested the involvement of alpha-adrenergic constriction and beta-adrenergic dilatation in the vascular effects of hydralazine. Possibly, several of these mechanisms are involved in the hemodynamic effects of hydralazine.The present study was not designed to further elucidate the cellular or subcellular mode of action of the classical vasodilators.

CHAPTER 5

HEMODYNAMIC EFFECTS OF CALCIUM ENTRY BLOCKERS IN CONSCIOUS SHR

5.1 Introduction

In recent years, calcium entry blockers (CEBs) have gained recognition in the treatment of hypertensive disease. Calcium entry blockers, such as verapamil and nifedipine, effectively lower blood pressure in hypertensive patients (Guazzi et al, 1977; Kiowski et al, 1983) and in various hypertensive animal models, including the SHR (Ishii et al, 1980; Lederballe Pedersen et al, 1982; Takata and Hutchinson, 1983). It is generally supposed that the blockade of calcium entry into vascular smooth muscle cells leads to a decrease in calcium dependent sympathetic or hormonal induced vascular tone, reducing vascular resistance. Recent clinical studies have indeed shown that the fall in blood pressure during therapy with nifedipine was associated with a fall in total vascular resistance (McLeay et al, 1983; Kiowski et al, 1983). Gross et al (1979) showed that nifedipine also reduced total vascular resistance in conscious normotensive dogs. A similar reduction in total peripheral resistance reductions was observed for several calcium entry blockers by Hof (1983) in anesthetized normotensive cats. Flaim and Zelis (1982) and Kanda et al (1984) observed a reduction of total peripheral resistance in conscious normotensive rats, following the calcium entry blockers diltiazem and nifedipine. Thus, mostly only normotensive animal models have so far been used to study the hemodynamic effects of calcium entry blockers. Since several authors have reported much more pronounced antihypertensive responses to calcium entry blockers in conscious SHR than in normotensive rats (Ishii et al, 1980; Takata and Hutchinson, 1983) it

seemed of interest to study the hemodynamic effects of calcium entry blockers in the hypertensive animal model.

In chapter 1, we summarized the regional hemodynamic effects of calcium entry blockers. In these studies, regional flow changes were mainly measured in normotensive conscious and anesthetized animals using the microsphere technique. Most of these studies show that calcium entry blockers decrease coronary, cerebral and muscular vascular resistance. In the other vascular beds, the regional effects are much more divergent in response to calcium entry blockers (see chapter 3).

The second purpose of the present study was to determine regional hemodynamic actions of the calcium entry blockers verapamil, nifedipine and PY 108-068. We also studied the effects of nifedipine on regional hemodynamics in WKY rats to check possible selectivity of CEBs for SHR. For this purpose, the rats were chronically instrumented with Doppler flowprobes to allow regional flow measurements.

We have previously shown that acute hemodynamic effects of antihypertensive agents as beta-blockers (Struyker Boudier et al, 1979) and classical vasodilators (see chapter 4 of this thesis) in conscious SHR are strongly influenced by sino-aortic baroreceptor reflexes. Barron et al (1983) reported that regional vasodilator responses to calcium entry blockers in normotensive rats were also strongly influenced by these reflexes. Therefore, as a third purpose of this study, we compared the regional hemodynamic effects of calcium entry blockers in intact and in sino-aortic baroreceptor denervated SHR to study interactions of the sino-aortic baroreflex with the effects of these drugs in SHR.

In the long-term, the influence of baroreceptor reflex mechanisms may be less dominant because of adaptation of these mechanisms to the prevailing level of blood pressure (Smits et al, 1982; Struyker Boudier, 1984). During long-term treatment, possibly the renin-angiotensin-aldosterone system could influence the regional hemodynamics of CEBs. Therefore, a last aspect of our study with CEBs was to investigate their effects upon chronic application. In these studies, long-term effects of verapamil on plasma renin concentration and regional hemodynamics were determined.

5.2 Materials and methods

5.2.1 Animals

Male SHR and normotensive Wistar Kyoto (WKY) rats, weighing 260-320 g, were used. More details are described in section 2.1.

5.2.2 Experimental protocol acute studies

Systemic hemodynamic effects of CEBs were determined in intact SHR. Regional hemodynamics were measured in intact SHR and WKY rats and in baroreceptor denervated SHR. Surgery and measurements for the central and regional hemodynamic studies are described in paragraph 2.3 and 2.4 respectively. The baroreceptor denervation was performed as described in section 2.6 of this thesis.

On the day of the experiments, the animals were placed in experimental cages between 9:00 and 10:00 a.m. They were allowed to habituate to the experimental conditions for at least 1 hr. Pre-injection values were obtained as the average of 4 readings at 5 min intervals in the last 20 min before an injection. Drugs were injected i.v. in 0.1 ml 0.9% NaCl (saline) in the case of verapamil or 0.1 ml of a mixture of polyethylene glycol (PEG), ethanol and 0.9% NaCl (v/v/v, 1/1/2) in the case of nifedipine and PY 108-068. Injections of solvent or different drug concentrations were given in random order. Hemodynamic variables were recorded continuously for at least 2 hr following injections. Effects were measured as differences from the pre-injection values.

5.2.3 Experimental protocol long-term studies

5.2.3.1 Regional hemodynamic measurements

In the long-term regional hemodynamic studies, surgery and measurements were performed as in the acute studies. However, the drug was not administered as a bolus injection via the femoral vein catheter, but as a continuous infusion using Alzet^{TM} osmotic minipumps via the jugular vein catheter. MAP, HR, RF, MF and HQF were measured every day (during $\pm$1.5 hr) from one day before until 5 days after the start of the infusion. The infusion was started by connecting the drug containing minipump under light ether anesthesia to the jugular vein

Table 5.1: Pre-injection absolute values (± SD) of central hemodynamic variables in the different experimental groups. Units: mean arterial pressure (MAP), mm Hg; cardiac index (CI), ml/min.100 g bw; total peripheral resistance index (TPRI), mm Hg.min.100 g bw/ml; heart rate (HR), bpm); stroke volume index (SVI), µl/100 g bw.

		N	MAP	CI	TPRI	HR	SVI
0.9% NaCl	0.1 ml	10	139±10	30.8±7.8	4.8±1.3	395±44	78±22
0.9% NaCl+PEG	0.1 ml (see Methods)	8	160±28	26.4±5.4	6.1±1.6	353±31	75±15
Verapamil	0.3 mg/kg	4	157±25	34.5±2.2	4.6±0.7	390±36	89±12
	1 mg/kg	7	157±14	35.0±4.0	4.6±0.8	403±28	87±10
	3 mg/kg	7	161±15	29.6±2.4	5.5±0.7	376±28	79± 9
Nifedipine	0.1 mg/kg	7	154±15	30.2±7.0	5.4±1.3	385±38	79±17
	0.3 mg/kg	6	159± 7	24.6±2.4	6.5±0.5	371±46	67± 9
PY 108-068	0.03 mg/kg	5	154± 5	29.2±1.0	5.4±1.0	395±34	74±12
	0.1 mg/kg	7	164±18	31.6±6.8	5.4±1.3	379±30	84±13
	0.3 mg/kg	5	153± 8	27.5±3.8	5.7±1.0	394±35	70±10

catheter and implanting it under the skin of the rat. The effects are expressed as percentage change from pre-infusion values on day 0.

5.2.3.2 Plasma renin concentration measurements

The SHR used in this study were implanted with an abdominal aorta catheter and a jugular vein catheter one day before the start of the experiment. Via the catheter in the abdominal aorta, MAP and HR were measured and blood samples were collected just before and after a 4 days infusion period. Verapamil (10 mg/kg.d) was administered via a jugular vein catheter using an Alzet™ minipump. The plasma renin concentration in these blood samples was quantitated as described in section 2.7.

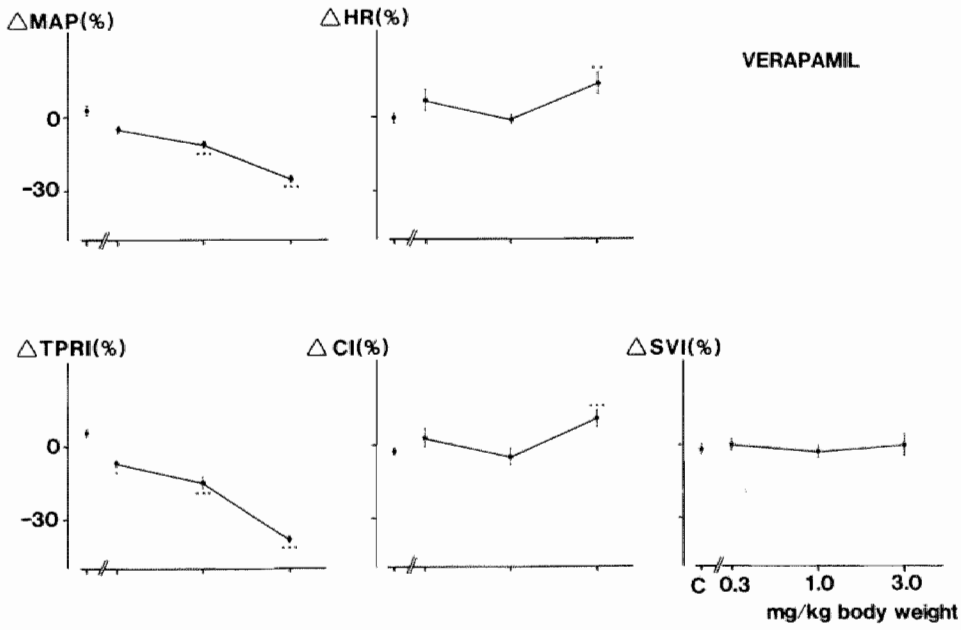


*Fig. 5.1: Effects of various doses of verapamil on mean arterial pressure (MAP), heart rate (HR), total peripheral resistance index (TPRI), cardiac index (CI), and stroke volume index (SVI) in conscious SHR. Significances are given in comparison to control injections (C) (*p<0.05; **p<0.01; ***p< 0.001; n=4-7).*

5.3 Results

5.3.1 Acute studies

5.3.1.1 Central hemodynamic studies

Pre-injection values for the different hemodynamic variables are summarized in table 5.1. The maximal effects of the three calcium entry blockers are shown in figs. 5.1-5.3. Saline and the solvent for nifedipine and PY 108-068 caused only minor hemodynamic changes.

All three agents caused a rapid fall in MAP and TPRI. These effects reached their maximum within 5-10 min after injection and the magnitude of the effects was dose-dependent. PY 108-068 was the most potent agent, causing a reduction in MAP of 36±2% and TPRI of 52±1% at a dose of 0.3 mg/kg. Nifedipine at a dose of 0.3 mg/kg caused a maxi-

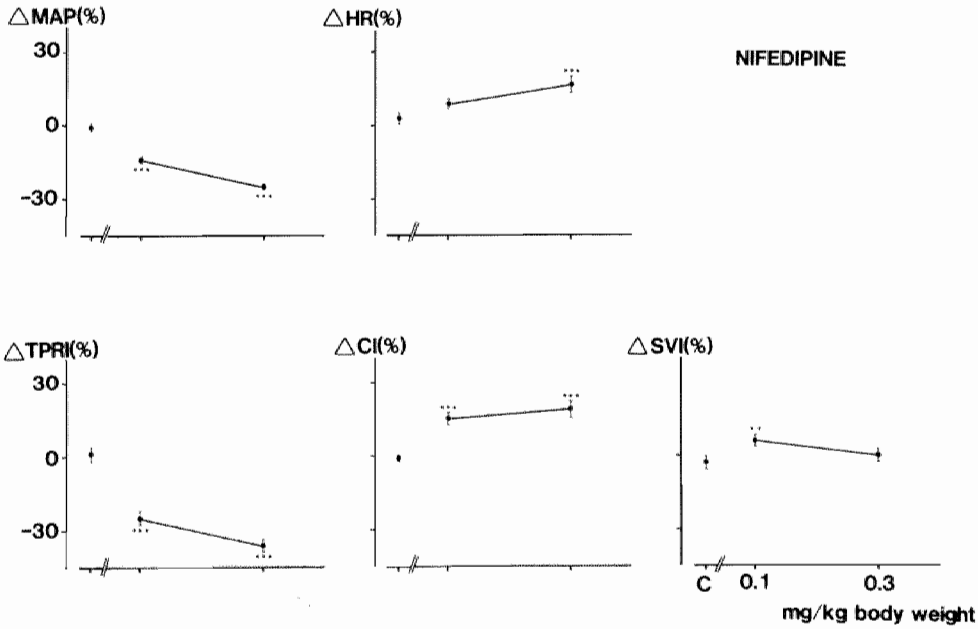


*Fig. 5.2: Effects of various doses of nifedipine on mean arterial pressure (MAP), heart rate (HR), total peripheral resistance index (TPRI), cardiac index (CI), and stroke volume index (SVI) in conscious SHR. Significances are given in comparison to control injections (C) (**p<0.01; ***p<0.001; n=6-7).*

mal MAP decrease of 25±1% and of 36±3% for TPRI. Similar effects were obtained at a dose of 3.0 mg/kg verapamil (MAP: -25±2%; TPRI: -38±1%). Depending upon the dose, the fall in MAP and TPRI lasted 0.5-2 hr.

The effects on HR and CI differed for the three calcium entry blockers. In the case of verapamil, no significant changes were observed in these variables after injection of 0.3 and 1.0 mg/kg. Only at the highest dose of 3.0 mg/kg significant ($p<0.01$) increases of 14±4% and 11±4% were observed for HR and CI. Nifedipine and PY 108-068 induced a rise in HR and CI at both doses (0.1 and 0.3 mg/kg) that caused a significant fall in MAP (figs. 5.2 and 5.3).

None of the three calcium entry blockers caused a significant change in SVI except for a small, but significant ($p<0.01$) increase (6±1.8%) after the lower dose of nifedipine (0.1 mg/kg, fig. 5.2).

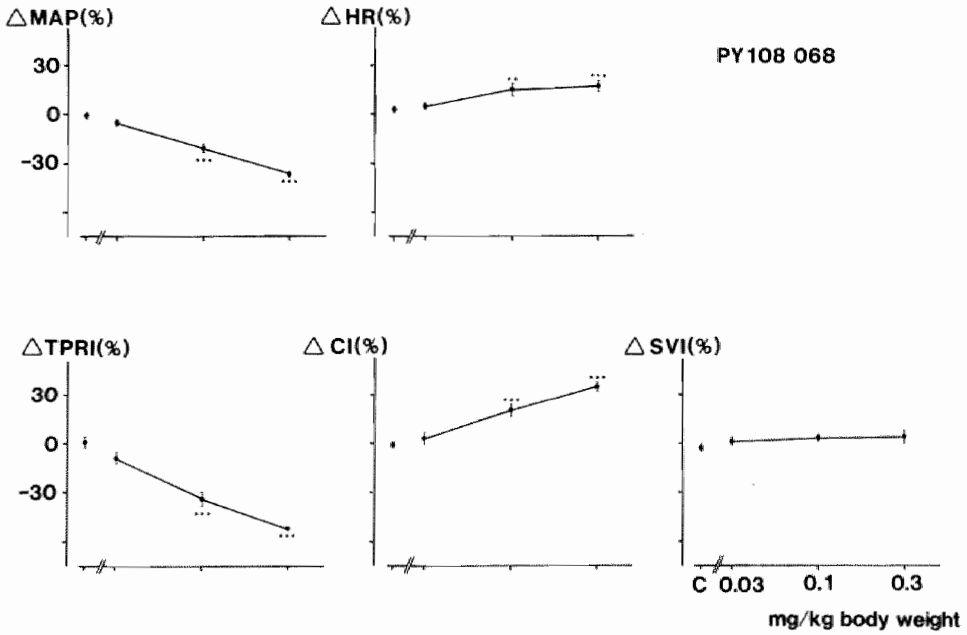


*Fig. 5.3: Effects of various doses of PY 108-068 on mean arterial pressure (MAP), heart rate (HR), total peripheral resistance index (TPRI), cardiac index (CI), and stroke volume index (SVI) in conscious SHR. Significances are given in comparison to control injections (C) (**p<0.01; ***p<0.001; n=5-7).*

Table 5.2: Pre-injection absolute values (± SD) of mean arterial pressure (MAP, mm Hg) and heart rate (HR, bpm) in the different experimental groups used in the regional hemodynamic experiments.

		N	MAP	HR
0.9% NaCl	0.1 ml	7	152±18	350±25
0.9% NaCl + PEG	0.1 ml (see Methods)	7	167±19	311±33
Verapamil	0.3 mg/kg	12	137±19	342±44
	1 mg/kg	14	132±16	326±41
	3 mg/kg	12	132±15	325±38
	10 mg/kg	11	128±15	335±36
Nifedipine	0.1 mg/kg	7	140± 9	343±48
	0.3 mg/kg	7	140±19	355±31
	1 mg/kg	8	130± 8	359±41
PY 108-068	0.01 mg/kg	4	163±14	328±40
	0.03 mg/kg	8	153±21	331±22
	0.1 mg/kg	8	146±19	330±32
	0.3 mg/kg	8	139±16	328±25
	1 mg/kg	7	139±17	336±34

5.3.1.2 Regional hemodynamic studies

Spontaneously hypertensive rats

Pre-injection values for MAP and HR for the different experimental groups are summarized in table 5.2 and the effects of the three calcium entry blockers on regional hemodynamics are shown in figs. 5.4-5.6. The three drugs caused a fall in MAP, of similar magnitude and duration as those in the systemic hemodynamic experiments. Verapamil was without effect on HR in these experiments also, even at the higher doses. Nifedipine and PY 108-068 caused a dose-dependent rise in HR, of similar magnitude and duration as those in the central hemodynamic experiments.

In spite of the profound blood pressure reductions, none of the calcium entry blockers decreased hindquarter blood flow (HQF). PY 108-068 even slightly increased HQF (maximally +9.6±4.1% after 0.3

mg/kg). Thus, all three calcium entry blockers reduced HQR in parallel to the fall in MAP. At the highest doses, the fall in HQR amounted to 20±5% for verapamil (10 mg/kg; $p<0.001$), 24±5% for nifedipine (1.0 mg/kg; $p<0.001$) and 24±5% for PY 108-068 (1.0 mg/kg; $p<0.001$). Vasodilation was maximal at 5-15 min after injection and lasted 0.5-2 hr, depending upon the dose injected. Mesenteric flow (MF) decreased in parallel to the fall in MAP. Thus, at the highest doses, the flow reductions in the mesenteric bed were 32±7% (verapamil), 11±5% (nifedipine), and 38±7% (PY 108-068). Calculation of MR showed that none of the drugs significantly altered this parameter. Nifedipine caused a consistent but non-significant reduction of MR (fig. 5.5). At low doses, verapamil (fig. 5.4) and PY 108-068 (fig. 5.6) did not change MR, whereas at higher doses, both drugs increased MR. A more complex

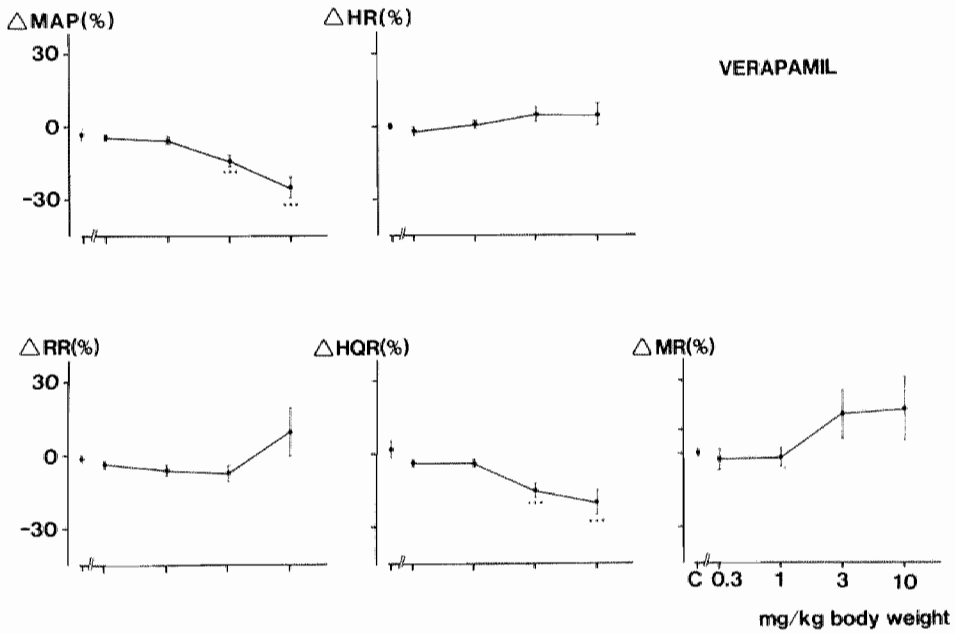


*Fig. 5.4: Effects of various doses of verapamil on mean arterial pressure (MAP), heart rate (HR), renal resistance (RR), hindquarter resistance (HQR), and mesenteric resistance (MR) in conscious SHR. Significances are given in comparison to control injections (C) (***$p<0.001$; $n=6-14$).*

picture was obtained for renal hemodynamics. Verapamil and nifedipine consistently lowered renal flow (RF), resulting in unchanged RR. At low doses (0.03 and 0.1 mg/kg), PY 108-068 did not influence RF, whereas these doses lowered MAP. Thus, RR was decreased by 13±4% ($p<0.01$) and 12±1% ($p<0.01$) after 0.03 respectively 0.1 mg/kg. This effect was no longer observed after the higher doses (fig. 5.6).

Normotensive Wistar Kyoto rats

In order to check for possible selectivity of the regional hemodynamic effects of calcium entry blockers for SHR, we studied the effects of nifedipine (0.1-1 mg/kg) and its solvent in a group of 6 WKY rats. Pre-injection MAP ranged from 105-120 mm Hg and HR from

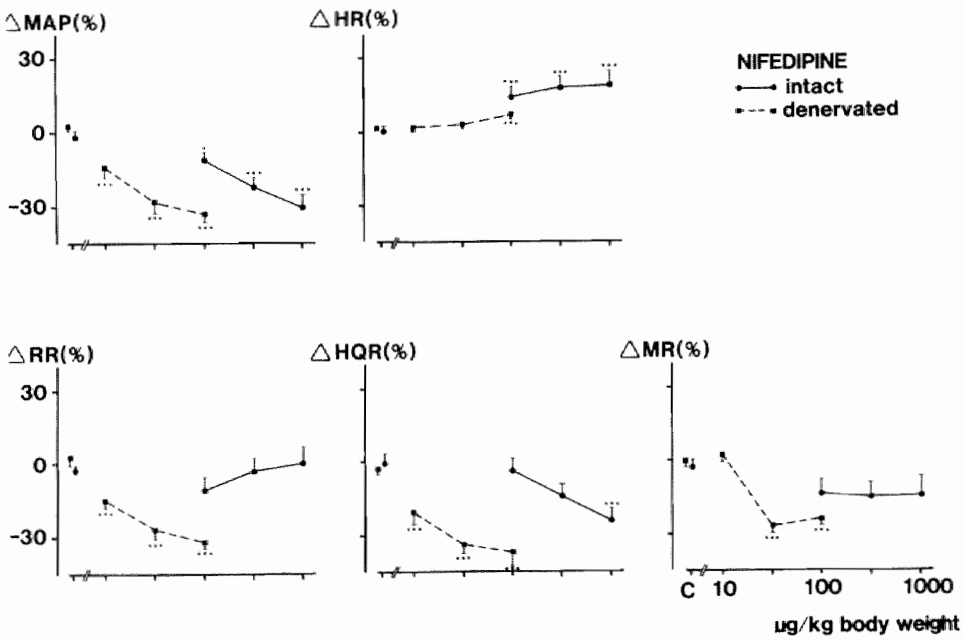


*Fig. 5.5: Effects of various doses of nifedipine on mean arterial pressure (MAP), heart rate (HR), renal resistance (RR), hindquarter resistance (HQR), and mesenteric resistance (MR) in conscious intact and sino-aortic denervated SHR. Significances are given in comparison to control injections (C) (***$p<0.001$; $n=6-8$).*

300-360 bpm/min in these animals. Nifedipine caused a dose-dependent fall in MAP with a maximum reduction of 22±2% ($p<0.001$; vs. -1±1% after solvent) and an increase in HR of 28±3% ($p<0.001$; vs. +2±2% after solvent) at a dose of 1 mg/kg At this dose, RF and MF decreased by 33±5% and 14±3%. On the other hand, HQF increased by 29±13%. Calculation of the resistance indicates a non-significant change in RR (+19±9%) and MR (-9±5%). HQR, on the other hand, was reduced significantly by -38±8% ($p<0.001$ vs. +2±3% after solvent).

5.3.1.3 Influence of sino-aortic baroreceptor denervation on regional hemodynamics

Since the regional hemodynamic effects of verapamil and nife-

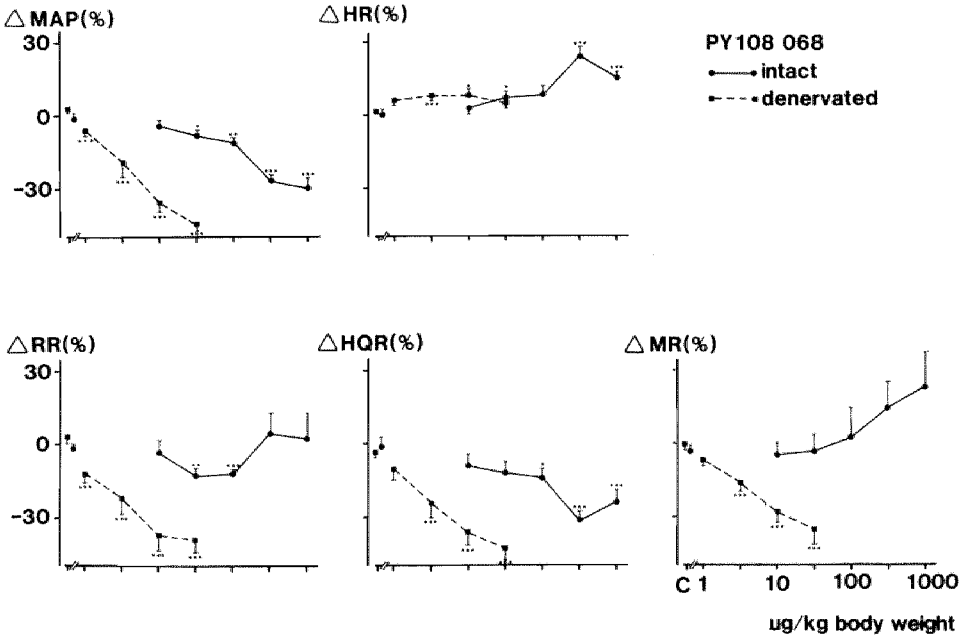


*Fig. 5.6: Effects of various doses of PY 108-068 on mean arterial pressure (MAP), heart rate (HR), renal resistance (RR), hindquarter resistance (HQR), and mesenteric resistance (MR) in conscious intact and sino-aortic denervated SHR. Significances are given in comparison to control injections (C) (*p<0.05; **p<0.01; ***p<0.001; n=4-8).*

dipine were very similar, we studied the influence of baroreceptor denervation only for nifedipine and PY 108-068. The pre-injection values for MAP and HR in the different groups used in this experiment are given in table 5.3.

Table 5.3: Pre-injection absolute values (± SD) of mean arterial pressure (MAP, mm Hg) and heart rate (HR, bpm) in the different sino-aortic denervated SHR used in the regional hemodynamic experiments.

		N	MAP	HR
0.9% NaCl + PEG (see Methods)		9	159±24	371±44
Nifedipine	10 µg/kg	7	166±28	392±56
	30 µg/kg	7	162±20	387±50
	100 µg/kg	7	142±19	393±31
PY 108-068	1 µg/kg	5	146±18	378±35
	3 µg/kg	6	156±32	354±15
	10 µg/kg	6	152±31	349±12
	30 µg/kg	6	148±33	344±10

Pilot experiments indicated that sino-aortic denervated SHR were much more sensitive than non-denervated animals to the blood pressure lowering effect of calcium entry blockers. Therefore, the range of doses investigated was 10-fold lower than that in the previous experiment. Again, both nifedipine (fig. 5.5) and PY 108-068 (fig. 5.6) caused a dose-dependent fall in MAP. The maximal effects in these animals occurred within 2 min after injection. When compared with the respective non-denervated groups (figs. 5.5 and 5.6), the dose-response curves for the maximal effects were shifted to the left on the dose axis by a factor of 10. The denervated animals showed only a small degree of tachycardia. For a comparable fall in MAP, the increase in HR was much less in the denervated animals (e.g. 1 mg/kg nifedipine in non-denervated SHR: MAP -30±5%; HR: +19±3% vs. 0.1 mg/kg in denervated SHR: MAP -32±3%; HR: +7±1%).

Regional hemodynamic measurements in baroreceptor denervated animals indicate a generalized vasodilation in parallel with the fall in MAP. The relative decrease in MAP corresponded closely to the fall in vascular resistance in the three vascular beds for all doses of nifedipine and PY 108-068.

5.3.2 Long-term studies

5.3.2.1 Effect on regional hemodynamics

Pre-infusion values of MAP (mm Hg) and HR (bpm) for the saline (0.9% NaCl) and the verapamil (10 mg/kg.d) treated groups, just before the start of the infusion on day 0, are summarized in table 5.4.

Table 5.4: Pre-infusion values of MAP (mm Hg) and HR (bpm) for the different experimental groups in the long-term regional hemodynamic study. Data are expressed as means ± SEM.

	n	MAP	HR
0.9% NaCl	13	155±3	315±11
Verapamil	10	157±3	337±5

There were no statistically significant differences between the pre-infusion values of MAP and HR in the two groups. Long-term hemodynamic effects on MAP, HR, renal hindquarter and mesenteric flow and the calculated resistances in the three vascular beds, expressed as percentage changes from the pre-infusion values are presented in fig. 5.7.

A 5-day infusion period of verapamil (10 mg/kg.d) reduced MAP significantly ($p<0.05$) as compared to the control group. During the first infusion day, MAP was reduced by 12±1% and remained reduced to a similar degree during the following 4 infusion days.

No influence of verapamil was observed on HR during long-term infusion. Also, no differences in renal, hindquarter and mesenteric flow were observed between the control and verapamil treated groups.

Renal and hindquarter resistances were reduced almost continuously during the first 3 infusion days. Mesenteric resistance was reduced during the first infusion day. The resistances in all three vascular beds remained reduced to a similar degree during the following infusion days. Although in the long-term the individual resistances were reduced by approximately 20% these effects did not reach a level that was statistically significantly different from the effects in the control group.

5.3.2.2 Effect on plasma renin concentration

The absolute values of MAP, HR and PRC measured on day 0 before the start and after a 4 days infusion period of saline and

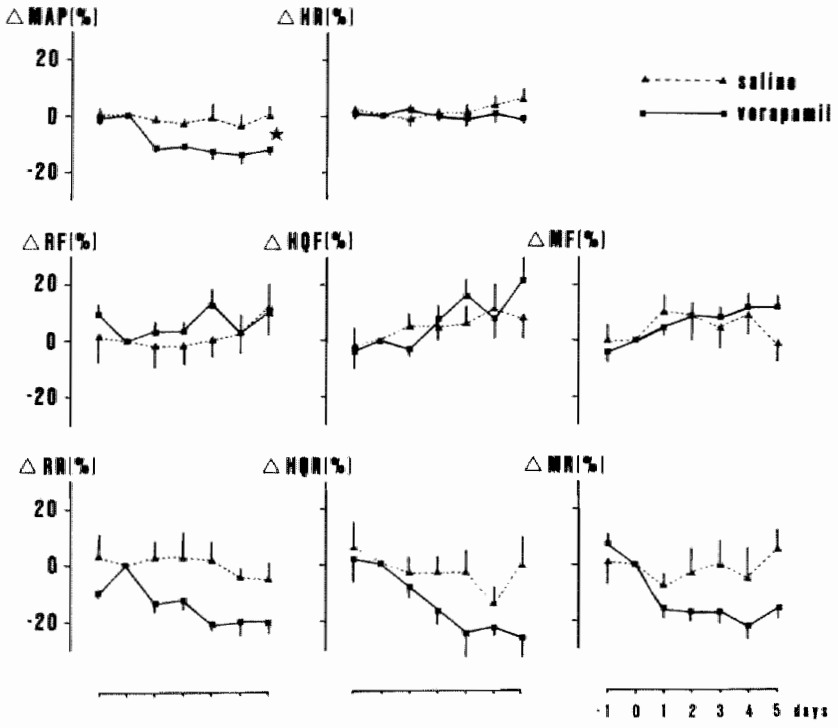


Fig. 5.7: Percentage changes (mean ± SEM) in mean arterial pressure (MAP), heart rate (HR), renal (RF, RR), hindquarter (HQF, HQR) and mesenteric IMF, MR) flow and resistance during a 5-day i.v. infusion of saline (24 µl/d), or 10 mg/kg.d verapamil in conscious SHR. Osmotic minipumps were implanted after the second measurement.

verapamil (10 mg/kg.d) are presented in table 5.5.

No significant differences were observed in the pre-infusion values of MAP, HR and PRC in the different experimental groups. Long-

*Table 5.5: Blood pressure (MAP, mm Hg), heart rate (HR, bpm) and plasma renin activity (PRC, ng AI/ml.hr) in the saline and verapamil (10 mg/kg.d) treated group just before (day 0) and after a 4-day infusion period. Data are expressed as mean ± SEM. Significances are given in comparison to pre-infusion values: **p<0.01.*

	n	day	MAP	HR	PRC
0.9% NaCl	6	0	176±7	325±7	7.1±0.8
(0.1 ml)		4	168±7	338±9	7.5±1.1
verapamil	11	0	175±3	330±6	7.3±1.1
(10 mg/kg.d)		4	152±3**	328±6	5.9±0.5

term infusion of verapamil (10 mg/kg.d) significantly reduced MAP by about 13% but did not influence HR. The MAP was significantly different from the control group. PRC was also reduced during long-term treatment although the difference was not significant.

5.4 Discussion

In this study, we investigated the central and regional hemodynamic actions of three calcium entry blockers in the conscious, unrestrained SHR. For the central hemodynamic measurements, rats were chronically instrumented with electromagnetic flowprobes and for the regional hemodynamics, with miniaturized Doppler flowprobes. In previous chapters discussed in this thesis, we were able to show the usefulness of these models to study hemodynamic effects. The major advantage of these models is that they allow the continuous characterization of hemodynamic effects of antihypertensive drugs in undis-

turbed, unanesthetized hypertensive animals.

The three calcium entry blockers used in this study caused a rapid and profound fall in blood pressure, the magnitude of which depended upon the dose injected. Moreover, we showed that the fall in blood pressure was associated with a reduction of total peripheral resistance. Previous studies indicated a similar hemodynamic pattern for various calcium entry blockers in hypertensive patients (Kiowski et al, 1983; Lehmann et al, 1983), normotensive dogs (Gross et al, 1979), cats (Hof et al, 1982) and rats (Flaim and Zelis, 1982).

In this study, we have paid special attention to the regional vascular effects underlying the fall in total peripheral resistance. Thus far, the effects of calcium entry blockers on regional blood flow distribution were mostly studied in normotensive rats and cats (see chapter 1) using the microsphere method. These studies show that different types of calcium entry blockers preferentially dilatate skeletal muscle, coronary and cerebral vascular beds. In other vascular beds (kidney, mesentery and skin), the effects of calcium entry blockers were more divergent. In our regional hemodynamic study, we observed a reduction in hindquarter but no change in renal and mesenteric resistance after verapamil and both dihydropyridines, PY 108-068 and nifedipine.

A reduction in muscular vascular resistance was observed by Reed and Tuma (1986) for nifedipine and Hof (1983) for PY 108-068 and nicardipine in anesthetized rats. Hof also observed a reduction in skeletal muscle vascular resistance for PY 108-068 and nifedipine in anesthetized cats. In another study, nisoldipine was found to have a vasodilator effect in skeletal muscle in conscious rats (Drexler et al, 1985). Kanda and Flaim (1984) observed in conscious rats only small reductions in muscular resistance. Flaim and Zelis (1982) showed that diltiazem reduces resistance in the skeletal muscle vascular bed. With respect to the hindquarter vascular bed, all these observations are in accordance with the present regional hemodynamic effects (However, a decrease in muscle vascular resistance together with no effect on renal and mesenteric resistance within one study was not observed in these previously published studies). Flaim and Zelis (1982) found no effect on renal but a reduction in mesenteric resistance. Kanda and

Flaim (1984) observed a resistance reduction in both the kidney and the mesentery and Drexler et al (1985) a reduction in renal resistance and no effect on mesenteric resistance. Even Barron et al (1983) who used the same method that we used in the present study found next to the profound reduction in hindquarter resistance also a decrease in mesenteric resistance after nitrendipine, nisoldipine and verapamil in normotensive rats. Furthermore, Bolt and Saxena (1984[b]) observed an almost general vasodilation for the calcium entry blocker felodipine in hypertensive rabbits. In the present study in hypertensive rats we did, however, only observe dilatation in the skeletal muscle.

The question arises whether these observations indicate that calcium entry blockers are vasodilators with selectivity for certain vascular beds, more specifically for the muscular bed. Our results in the sino-aortic denervated SHR point to a different explanation. Calcium entry blockers loose their predominant muscular vasodilating effect after baroreflex denervation. In fact, in the denervated SHR the degree of vasodilatation was comparable for all three vascular beds. The blood pressure lowering potency of the calcium entry blockers was increased 10-fold. In denervated conscious normotensive rats with verapamil, nitrendipine and nisoldipine (Barron et al, 1983) there was also a 10-fold increase in potency for these drugs. These results suggest that in intact animals the fall in blood pressure triggers a baroreceptor reflex mediated increase in sympathetic nerve activity which counteracts the direct vasodilatation otherwise observed in the renal and mesenteric vascular beds.

Other studies support the possibility of an acute activation of baroreflex mechanisms. Thus, several authors observed an early increase in plasma noradrenaline concentrations in hypertensive patients, following calcium entry blockers (Kiowski et al, 1983; Murphy et al, 1982).

In chapter 3 we have shown that a baroreflex activation induced by an unilateral carotid occlusion in conscious SHR increases renal, hindquarter and mesenteric resistance. Also the influence of the baroreflex on the effects of hydralazine (see chapter 4) on renal, hindquarter and mesenteric resistance was similar in all three vascular beds. Baroreceptor denervation did not affect the regional hemo-

dynamic response to this agent. These results indicate that preferential muscular vasodilatation of CEBs can not be explained on the basis of a different degree of sympathetic innervation of the vascular beds. This implies a possibly selective interference with the sympathetic influence on skeletal muscle vascular beds by CEBs. In our hemodynamic studies, drugs were administered i.v., so the site of action of the calcium entry blockers can be anywhere in the baroreflex pathway.

Heesch et al (1983) observed direct effects of calcium entry blockers on baroreceptor discharge in dogs. They presented evidence that nifedipine increased whereas verapamil decreased baroreceptor discharge. In that study, nifedipine and verapamil also had opposite effects on the renal sympathetic outflow. It is unlikely that such a direct effect of calcium entry blockers on baroreceptors explains the blockade of the sympathetic influence on the skeletal muscle vascular bed because in the present study, no differences beween nifedipine and verapamil were observed with regard to regional vascular beds. Furthermore, Kunze et al (1986) did not find direct effects of calcium entry blockers on baroreceptors in the range in which effects are expected to be selective for calcium entry blockade. Only in the range of high concentrations in which non-selective effects are expected did they observe a similarly depressed afferent reflex for nifedipine and verapamil.

A recent report from Higuchi et al (1985) suggests that in rats, calcium ions play an important role in the integral function of neurons in the brain stem, particularly in the nucleus tractus solitarii. They showed that verapamil, diltiazem and nifedipine administered in the brain stem produce excitation of the nucleus tractus solitarii neurons resulting in a withdrawal of sympathetic outflow. This could be a possible explanation for the observed regional hemodynamic effects of calcium entry blockers in the present study. In this hypothesis calcium entry blockers may block the sympathetic influence on the skeletal muscle possibly by a direct effect on the nucleus tractus solitarii without interfering the sympathetic outflow to the kidney and mesentery. In an unpublished study, we observed such a differentiated sympathetic outflow by electrical stimulation of the median raphe nucleus which is also involved in the baroreflex pathway.

It has been proposed that vasoconstriction mediated by $alpha_2$-adrenoceptors is dependent on the influx of extracellular calcium whereas the vasoconstriction mediated by $alpha_1$-adrenoceptors is not (Van Meel et al, 1983; Saeed et al, 1983; Van Zwieten et al, 1983). The non-selective catecholamines stimulate both $alpha_1$- and $alpha_2$-adrenoceptors at postsynaptic sites. Assuming that vascular tone is maintained by stimulation of both receptor subtypes the constrictor effect of $alpha_2$-adrenoceptor stimulation is diminished in the presence of a calcium entry blocker. Then, differences in $alpha_1/alpha_2$ ratio between the hindquarter and the other two renal and mesenteric vascular beds could also explain the selective blockade of the sympathetic influence on skeletal muscle vasculature as was seen in the present study. However, studies performed with perfused dog hind limbs showed that especially $alpha_1$-adrenoceptors are sympathetically innervated whereas $alpha_2$-adrenoceptors are located extrajunctionally (Langer and Shepperson, 1982). These studies suggest that a noradrenergic nerve stimulation of the skeletal muscle vascular bed will be relatively insensitive to inhibition by CEBs which makes the explanation based on the $alpha_1/alpha_2$ ratio very unlikely.

The present regional hemodynamic studies show that calcium entry blockers are not preferentially muscular vasodilators but also reduce renal and mesenteric resistance in baroreceptor denervated animals. Furthermore, it is likely that they also dilate other vascular beds to a considerable extent because the reduction in total peripheral resistance seen in the central hemodynamic studies was greater than the fall in resistance in the muscular bed. Possibly, coronary and cerebral resistances are also reduced although the methods we used do not allow quantitation of these resistances.

The baroreflex mediated increase in sympathetic activity explains some of the acute changes observed in central hemodynamic variables after calcium entry blockers. The early rise in heart rate and cardiac output following nifedipine and PY 108-068 were probably due to indirect nervous reflex mechanisms rather than a direct cardiac action of these drugs. A similar conclusion was reached by Nakaya et al (1983) in a recent study in which they investigated the cardiac responses to verapamil, diltiazem and nifedipine in chronically in-

strumented conscious dogs. The direct cardiac action of calcium antagonists may even lead to negative inotropic (nifedipine: Pérez et al, 1982) or negative chronotropic and inotropic (verapamil and diltiazem: Pérez et al, 1982; Nakaya et al, 1983) effects. The direct cardiac effects of verapamil may explain why increases in heart rate and cardiac output were almost completely absent in our study, following this drug in contrast to nifedipine or PY 108-068. In addition, the inhibitory effects of verapamil on carotid sinus baroreflex, as opposed to the excitatory effects of nifedipine may be involved in the differential effects of these agents on cardiac hemodynamic variables (Heesch et al, 1983).

In short-term studies, the baroreceptor reflex in particular may strongly influence the hemodynamic effects of an antihypertensive agent. On the long-term the influence of baroreceptor reflex mechanisms may be less dominant, because of adaptation of these mechanisms to the prevailing level of blood pressure. During long-term treatment, verapamil reduced renal hindquarter and mesenteric resistance. However, the effects on regional resistances during long-term verapamil treatment were not significantly different from the control values. This in contrast to the rapid significant reduction in blood pressure to a new steady-state level. Further studies will be necessary to find out whether higher infusion doses of verapamil administered to SHR will lead to a significant vasodilation in all three vascular beds.

If we assume that verapamil at higher doses induces a general vasodilatation during long-term treatment, this long-term regional pattern is similar to that observed in sino-aortic baroreceptor denervated animals in the acute studies. These results then suggest a rapid baroreceptor reflex resetting during long-term verapamil treatment. Mostly, baroreflex activity is correlated to the changes in heart rate and cardiac output. This is not possible in the case of verapamil because that drug directly interferes with the baroreflex pathway (Pérez et al, 1982; Heesch et al, 1983; Nakaya et al, 1983) reducing the baroreflex mediated effects on the heart. Thus, it is not clear whether the general reduction in vascular resistance is a consequence of a rapid baroreceptor resetting in the long-term studies.

In the long-term, the influences of baroreflex mechanisms may

be less dominant because of adaptation of this mechanism to the prevailing level of blood pressure. Then, possibly the renin-angiotensin-aldosteron system could influence the regional hemodynamics of calcium entry blockers. Therefore, we investigated in the last part of the study the influence of a long-term verapamil treatment on plasma renin activity in conscious SHR.

Our results showed a slight but not significant reduction in plasma renin concentration after a 4-day verapamil infusion. In contrast to the present findings, Dietz et al (1983) reported an increase in renin secretion during intrarenal infusion of nifedipine in anesthetized dogs. Similar results were observed by Imagawa et al (1986) and Abe et al (1983) in anesthetized dogs using intrarenal nifedipine infusions. Only at hypotensive doses of nifedipine, increases in renin secretion were observed by Dietz et al (1983). Furthermore, he did not observe an increase in renin secretion when the kidney was denervated. This in contrast to Imagawa et al (1986) who observed a stimulatory effect of nifedipine on renin release also at non-hypotensive doses. The results of Dietz et al suggest an involvement of the baroreflex mediated sympathetic activation on the kidney increasing renin secretion. The results of Imagawa et al (1986) suggest, however, a direct effect of calcium entry blockers on juxtaglomerular cells. Kotchen et al (1974) and Watkins et al (1976) have suggested that enhancing calcium influx into the juxtaglomerular cells inhibits the renin release from the juxtaglomerular cells. Taking this into consideration, the lack of an enhancement of renin secretion by verapamil during long-term treatment is possibily a consequence of blockade of sympathetic activation by a direct effect of verapamil on the juxtaglomerular cells, such an explanation is possible if the baroreflex is still not reset after 4 days of infusion, this is still unclear. Another possibility could be that the plasma verapamil concentration during intravenous infusion is too low in the present study for a pronounced direct effect on the juxtaglomerular cells as compared to the intrarenal nifedipine infusion.

In summary, the central and regional hemodynamic actions of the calcium entry blockers verapamil, nifedipine and PY 108-068 were evaluated in chronically instrumented, conscious SHR. All three agents

caused a dose-dependent fall in blood pressure and total peripheral resistance. The fall in blood pressure triggered a baroreflex mediated rise in heart rate and cardiac output which was probably counteracted by direct cardiac effects in the case of verapamil. In intact animals, the acute fall in total peripheral resistance was related primarily to a decrease in vascular resistance of the muscular bed. However, the calcium entry blockers cannot be regarded as selective dilators of this vascular bed, since in baroreflex denervated SHR and during long-term treatment in intact SHR, the degree of vasodilatation was similar in all three vascular beds studies. Also no effect on PRC was observed during chronic verapamil treatment.

CHAPTER 6

HEMODYNAMIC EFFECTS OF THE BETA-ADRENOCEPTOR BLOCKERS PROPRANOLOL AND TERTATOLOL IN CONSCIOUS SPONTANEOUSLY HYPERTENSIVE RATS

6.1 Introduction

Beta-adrenoceptor blockers are used in the therapy of a wide variety of cardiovascular diseases, including hypertension. The acute hemodynamic actions of most beta-adrenoceptor blockers in hypertensive patients or animals consist of a fall in cardiac output, whereas a rise in total peripheral resistance prevents an early fall in blood pressure (Ulrych et al, 1968; Smits et al, 1982; Cofler et al, 1984; reviews: Fitzgerald, 1984; Van Baak et al, 1985).

During the last few years, attempts have been made to develop beta-adrenoceptor blockers with ancillary vasodilator properties, e.g. prizidilol (Taylor et al, 1981) and carvedilol (Eggertsen et al, 1984). Furthermore, beta-blockers with a more selective regional hemodynamic profile of action have been described. In this respect, the development of beta-blockers that maintain normal renal perfusion have received special attention. Such an action has been claimed for the beta-blockers nadolol (O'Connor et al, 1982; Danesh et al, 1984) and tertatolol (Lantz et al, 1984).

The first purpose of the present study was to investigate the effect of the classical beta-blocker propranolol and tertatolol (Servier compound 2395) a potent, long-acting, non-selective beta-blocking agent without sympathomimetic activity (Laubie et al, 1973), on central and regional hemodynamics in conscious SHR.

Microsphere studies in several animal models indicate a generalized acute vasoconstriction following propranolol (Nies et al, 1973; Van Boom and Saxena, 1983; Hatzinikolaou et al, 1983).

In previous studies, it was shown that the early rise in total peripheral resistance is most likely caused by a baroreflex mediated

rise in sympathetic nerve activity (Struyker Boudier et al, 1979).

The second purpose of the present study was to further investigate the role of baroreceptor reflex activation in the early peripheral vasoconstriction following beta-adrenoceptor blockade with the beta-blockers propranolol and tertatolol. Therefore, we compared the regional hemodynamic effects determined with the Doppler technique of both beta-blockers in intact and sino-aortic baroreceptor denervated conscious SHR.

The third aspect on which this study focusses, is the effect of both beta blockers on renal hemodynamics and excretory function. It has been reported that renal plasma flow and/or glomerular filtration rate (GFR) are reduced during administration of beta-blockers (Wilkinson, 1982, Weber et al, 1984). However, nadolol has been reported to spare or even increases renal blood flow (Britton et al, 1981 and Danesh et al, 1981) without affecting GFR. Laubie et al (1986) showed that tertatolol and the classical beta-blocker propranolol have different effects on renal hemodynamics and function in conscious dogs. They found that, in contrast to propranolol, tertatolol increases GFR and urine and sodium excretion. In their study, no effect of tertatolol and propranolol on renal plasma flow was observed. Lantz et al (1984) observed that tertatolol given orally increases both renal plasma flow and GFR in hypertensive patients with or without chronic renal failure. These experiments suggest that tertatolol, like nadolol, preserves renal perfusion possibly by an additional pharmacodynamic action that is independent of beta-adrenoceptor blockade.

Baroreflex desactivation most likely causes the early increase in peripheral resistance after beta blockers, but during long-term treatment baroreflex resetting may occur and the influence of the renin-aldosteron system may be more dominant. Therefore as a fourth aspect of this study we investigated the long-term effects of both beta blockers on regional hemodynamics and plasma renin concentration in conscious SHR.

6.2 Experimental protocol

6.2.1 Animals

Male SHR, weighing 250-350 g, were used in the studies described in this chapter. More details are given in section 2.1.

6.2.2 Central hemodynamic studies

Methods for implantation of flow probes and catheters for central hemodynamic studies were described in chapter 2. For measurement of blood pressure and cardiac output, rats were placed in normal experimental cages (20x20x30 cm). After a stabilization period of 1 hr, an injection of 0.1 ml saline (n=10) or 0.5 mg/kg tertatolol (n=6) was given i.v. Hemodynamics were monitored continuously during the first 6 hr after the injection. Furthermore, measurements were made 20 hr after the injection. Cardiac output was normalized for body weight and expressed as ml/min.100 g bw. This value wil be referred to as cardiac index (CI). From MAP and CI, total peripheral resistance was calculated (TPRI = MAP/CI; mm Hg.min.100 g bw/ml). Furthermore, SVI was calculated from CI and HR (SVI = CI/HR; ml/100 g bw).

6.2.3 Regional hemodynamic studies

Two groups of animals were used for this experiment. In one group, the sino-aortic baroreceptors were denervated and in the other group the sinoarotic baroreflex was left intact. On the morning of the experimental day, the implanted measuring devices in the conscious animals were connected to their respective equipment. Arterial blood pressure was measured from the intra-aortic catheter using a miniature strain gauge transducer (model CP-01; Century Technology Company, Inglewood, Ca, USA). Regional blood flows were measured as KHz Doppler shift using a 4-channel 20-MHz directional pulsed Doppler system (Bioengineering Department, University of Iowa, Iowa City, IA, USA). Zero blood flow was determined electronically. Mean pressure and flow signals were obtained by electronic low-pass filtering. Regional resistance changes were calculated from pressure and flow changes according to procedures previously described in detail (Smits and Struyker Boudier, 1984).

Animals were given at least 1 hr to get used to the experimental conditions. Pre-injection values were obtained as the average of 4 readings at 5-min intervals in the last 20 min before an injection. Drugs were injected intravenously in 0.1 ml 0.9% NaCl after which the catheter was flushed slowly with 0.3 ml 0.9% NaCl. Control injections consisted of 0.1 ml 0.9% NaCl followed by slow infusion of 0.3 ml 0.9% NaCl. Injections of vehicle or drug solutions were given in random order with at least 2 days between injections in the same animal. Hemodynamic variables were recorded continuously for at least 4 hr following injection. Effects were measured as the difference from the pre-injection value.

6.2.4 Renal hemodynamic and excretory function studies

In this study, 4 experimental SHR groups were used receiving saline (0.1 ml), 5 mg/kg propranolol, 0.1 mg/kg or 0.5 mg/kg tertatolol respectively. GFR and ERPF were measured as plasma clearances of respectively [^{51}Cr] EDTA and [^{125}I]PAH in conscious SHR as described in paragraph 2.8 "Method B". Blood and urine samples were collected 1/2 hr before (1 sample/15 min) and 3 hr after (1 sample/30 min) the bolus injections of the respective drugs. GFR (ml/min.g kw) and ERPF (ml/min.g kw) were calculated from urine and plasma concentrations of [^{51}Cr] EDTA and [^{125}I]PAH. Filtration fraction (FF) was calculated as GFR/ERPF. The urine production (V) was continously and directly determined via a bladder catheter and expressed as µl/min.g kw.

6.2.5 Long-term regional hemodynamic studies

The same experimental protocol was performed to determine long-term regional hemodynamics as described in chapter 5.2.3.1. Regional hemodynamics were measured every day (during ca. 1.5 hr) one day before to 5 days after the start of the infusion of 5 mg/kg.d propranolol, 0.5 mg/kg.d tertatolol or saline using Alzet^{TM} minipumps. The effects are expressed as percentage change (mean $\pm$ SEM) from pre-infusion values measured on day 0.

6.2.6 Long-term plasma renin concentration studies

The plasma renin concentration studies were performed fol-

lowing the same experimental protocol as descirbed in chapter 5.2.3.2. Chronic infusion of saline, 5 mg/kg.d propranolol or 0.5 mg/kg.d tertatolol were performed using Alzet™ minipumps. The effects of MAP (mm Hg), HR (bpm) and PRC (ng AI/ml.hr) were expressed as means ± SEM.

6.3 Results

6.3.1 Effects on central hemodynamics

In this study, the effects of saline and tertatolol on central hemodynamics were determined. Baseline values for the different experimental groups used in the central hemodynamic studies are summarized in table 6.1.

Table 6.1: Absolute values (± SEM) of central hemodynamic variables before drug administration in the different experimental groups. Units: mean arterial pressure (MAP), mm Hg, cardiac index (CI), ml/min.100 g bw; total peripheral resistance index (TPRI), mm Hg.min.100 g bw/ml; heart rate (HR), bpm; stroke volume index (SVI), µl/100 g bw.

	0.9% NaCl (n=10) 0.1 ml	Tertatolol (n=6) 0.5 mg/kg
MAP	139±3	150±5
HR	395±14	365±14
CI	31±3	34±3
TPRI	4.8±0.4	4.6±0.4
SVI	79±7	91±6

There were no statistically significant differences between the pre-injection values.

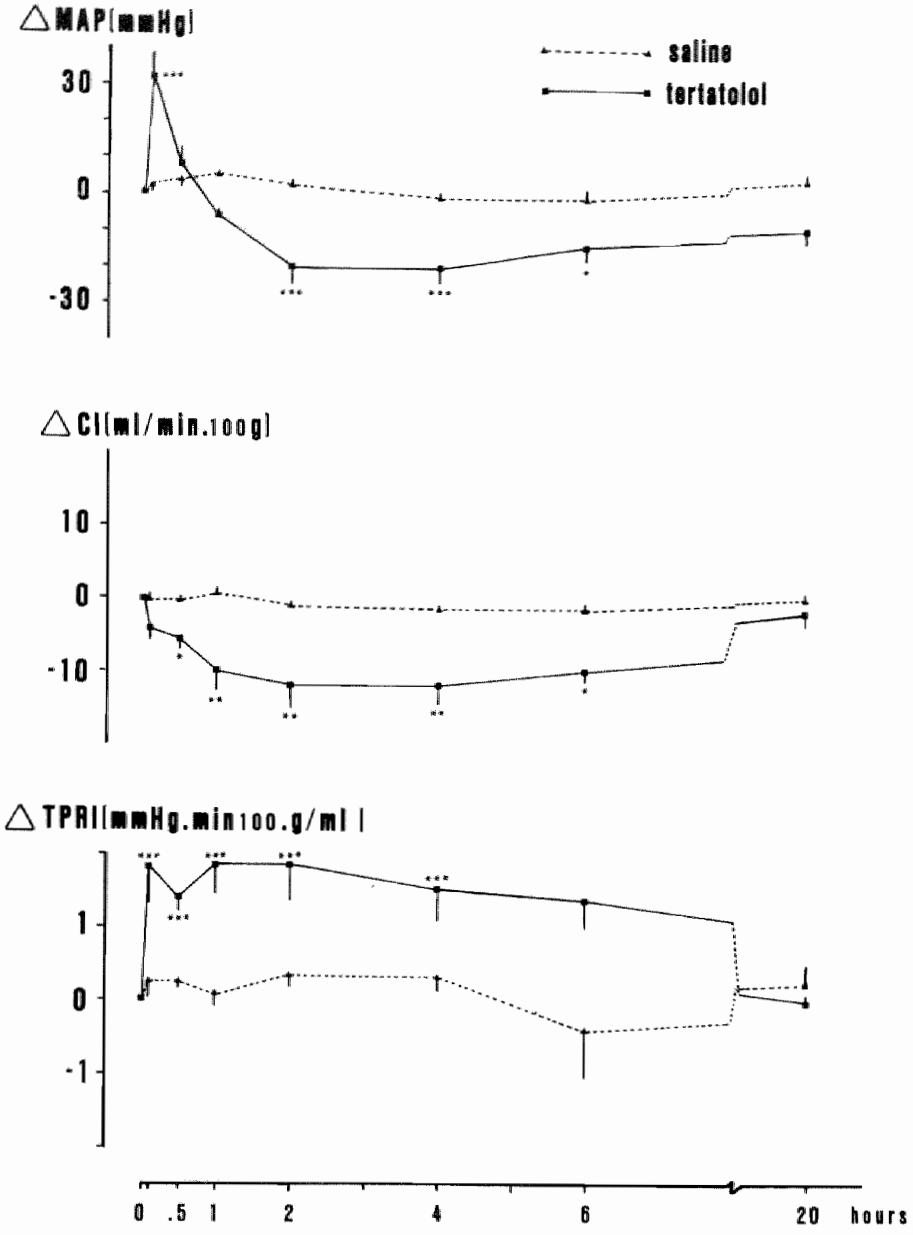


Fig. 6.1: Changes (mean + SEM) in mean arterial pressure (MAP; mm Hg), cardiac index (CI; ml/min.100 g bw) and total peripheral resistance index (TPRI; mm Hg.min.100 g.ml^{-1}) following saline (24 µl/d) or 0.5 mg/kg tertatolol in conscious SHR (n=6).

Effect of saline and 0.5 mg/kg tertatolol on MAP, CI and TPRI during 20 hr post-injection are presented in fig. 6.1 and on HR and SVI in fig. 6.2. Injection of saline caused only slight variations in MAP, CI and TPRI during the measuring periods. After tertatolol, two distinct phases in the effect on MAP could be recognized. During the first 1/2 hr, it significantly increased from baseline value by maximally 32±7 mm Hg ($p<0.001$) After that period, MAP decreased and was significantly below control values during 2 to 6 hr after the injection (maximal reduction 20±4 mm Hg; $p<0.001$). Twenty hr after

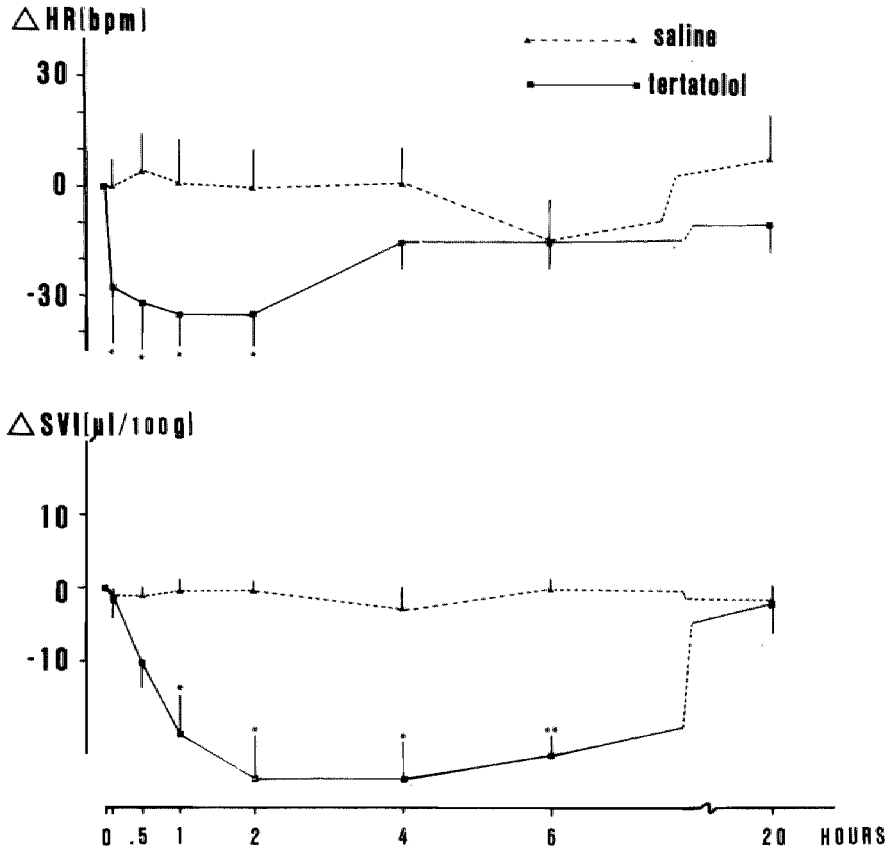


Fig. 6.2: Changes (mean ± SEM) in heart rate (HR; bpm) and stroke volume index (SVI; ml/100 g bw) following saline or 0.5 mg/kg tertatolol in conscious SHR (n=6).

tertatolol injection, MAP was still reduced but no longer significantly different from the control values. Tertatolol immediately reduced CI. This variable was significantly different from control values between 1/2 and 6 hr post-injection, with a maximal reduction of 12±2 ml/min.100 g bw ($p<0.01$) observed between 1 to 4 hr after the bolus injection.

HR was immediately and significantly reduced ($p<0.05$) after tertatolol to nearly the same extent from 1/2 to 2 hr (maximal reduction: -35±9 bpm). Four hr post-injection, HR returned to control values. SVI also decreased immediately after the bolus injection of tertatolol. One hour after injection SVI was significantly reduced with a maximum of -28±5 µl/100 g; $p<0.05$). It returned to control values within 20 hr post-injection.

Injections of 0.5 mg/kg tertatolol caused an immediate steep increase in TPRI. It was significantly different from control values ($p<0.001$) between 1/2 and 4 hr post-injection (maximal increase: 1.8±0.4 mm Hg.min.100 g bw/ml). Twenty hr post-injection, no statistically significant differences in MAP, CI and TPRI were observed between values of the drug treated and control groups.

6.3.2 Effect on regional hemodynamics

6.3.2.1 Intact animals

Table 6.2 summarizes the pre-injection values in the different experimental groups and figures 6.3-6.5 summarize the regional hemodynamic effects of injections of 0.9% NaCl (n=7), 5 mg/kg propranolol (n=9) and 0.5 mg/kg tertatolol (n=7), measured at 15-30 min and 3-4 hr post-injection. Both beta-blockers caused an immediate fall in HR, which was maximal 15-30 min after injection. MAP, on the other hand, changed in a biphasic manner. During the first 15-30 min after the beta-blockers, it increased significantly, whereas it fell below control values within 3-4 hr after injection.

Propranolol caused a significant early (15-30 min) increase in the resistances of the renal, mesenteric and hindquarter vascular beds. This increase was parallelled by a fall in blood flow through all tissues, although a statistically significant level was attained only for renal blood flow.

*Table 6.2: Pre-injection values (mean ± SEM) of MAP(mm Hg) and HR (bpm) in intact and sino-aortic baroreceptor denervated (SAD) SHR. *p<0.05: significantly different from intact animals. n = number of test animals.*

Experimental group	n	MAP	HR
NaCl (0.9%)			
Intact	7	147±8	303±9
SAD	10	143±10	354±12*
Propranolol (5 mg/kg)			
Intact	9	140±6	304±5
SAD	10	154±7	374±8*
Tertatolol (0.5 mg/kg)			
Intact	7	141±5	301±8
SAD	10	136±5	374±11*

A different pattern of regional hemodynamic changes was obtained after tertatolol. This agent caused a significant early increase in HQR only. Renal resistance gradually decreased to reach a statistically significant reduction 3-4 hr after injection. Renal blood flow remained stable and even tended to increase although not significantly.

6.3.2.2 **SAD animals**

The effects of 0.9% NaCl (n=10), 5 mg/kg propranolol (n=10) and 0.5 mg/kg tertatolol (n=10) in SAD animals are summarized in figs. 6.3-6.5. Table 6.2 shows the pre-injection values of MAP and HR in intact and SAD animals. The data show that SAD does not lead to a further elevation in blood pressure in conscious SHR, although we noted that blood pressure was much more labile in these animals. On the other hand, HR was significantly higher in the SAD animals.

Both propranolol and tertatolol caused a larger and longer lasting fall in the HR in SAD than in intact SHR (fig. 6.3). Furthermore, MAP decreased almost immediately after the beta-blocker administration in SAD animals. It was already significantly lowered at 15-30 min. After 3-4 hr, the reduction in MAP did no longer differ significantly from that in intact animals.

In SAD animals, propranolol and tertatolol no longer caused a significant early increase in resistance in the mesenteric and hindquarter vascular beds. The respective blood flows decreased significantly 15-30 min after the administration of propranolol and tertatolol. In the hindquarter vascular bed, the flow reduction persisted until 3-4 hr after injection of both beta-blockers.

In the renal vascular bed, the early increase in resistance which occurred only with propranolol in intact animals, was no longer observed in SAD animals. Renal resistance even decreased with both

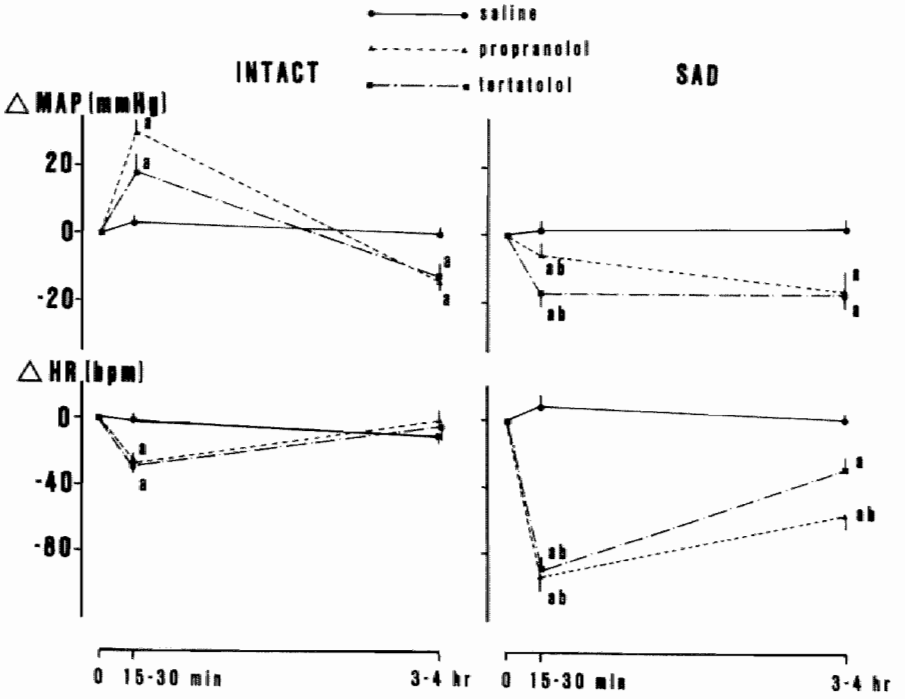


Fig. 6.3: Effects of saline, 5 mg/kg propranolol and 0.5 mg/kg tertatolol on mean arterial pressure (MAP: mm Hg) and heart rate (HR: bpm) in intact and sino-aortic baroreceptor denervated (SAD) conscious SHR.
[a] significantly ($p<0.05$) different from corresponding saline group
[b] significantly ($p<0.05$) different from corresponding intact rats.

agents at 15-30 min and 3-4 hr after administration.

6.3.3 Effects on renal hemodynamics and excretory function

Effects on renal plasma flow (EPRF), glomerular filtration rate (GFR), filtration fraction (FF) and urine production (V) in the 4

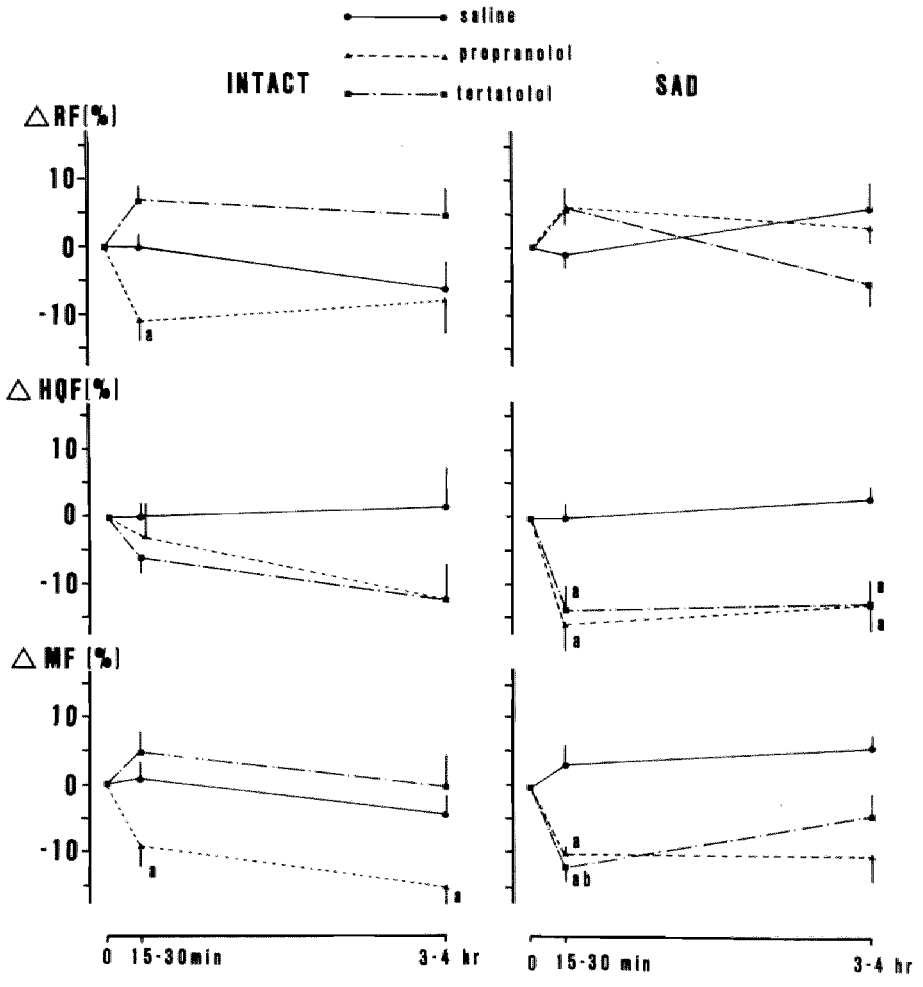


Fig. 6.4: Percentage change of renal blood flow (RF), hindquarter flow (HQF) and mesenteric flow (MF) after i.v. saline, 5 mg/kg propranolol or 0.5 mg/kg tertatolol administration in intact and sino-aortic baroreceptor denervated (SAD) conscious SHR.

different experimental groups measured -15 to 0 min before and 30 to 60 min and 120-150 min after the injection expressed in absolute values are summarized in table 6.3.

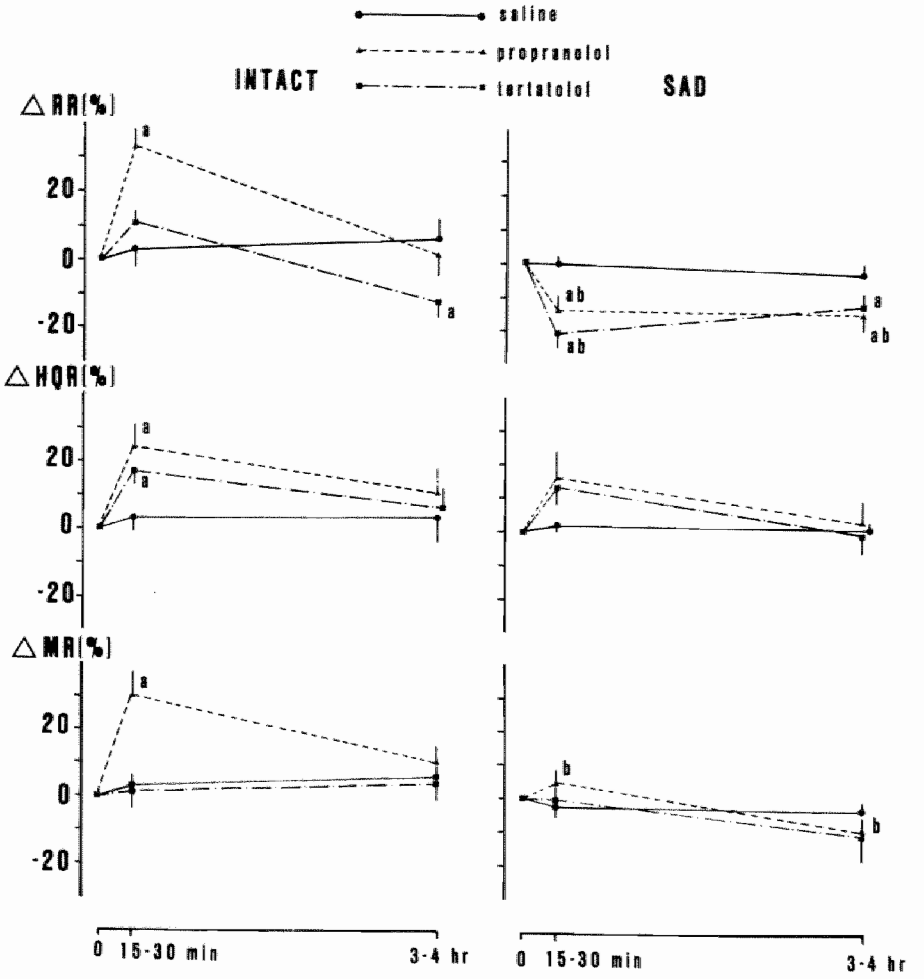


Fig. 6.5: Percentage change of renal blood resistance (RR), hindquarter resistance (HQR) and mesenteric resistance (MR) after i.v. saline, 5 mg/kg propranolol or 0.5 mg/kg tertatolol administration in intact and sino-aortic baroreceptor denervated (SAD) conscious SHR.

*Table 6.3: Effects of intravenous injections of saline (0.1 ml), propranolol (5 mg/kg), tertatolol (0.1 mg/kg and 0,5 mg/kg) on ERPF (ml/min.g kw), GFR (ml/min.g kw), FF and V (µl/min. g kw). Data are means ± SEM. *p<0.05; **p<0.01: significantly different from control values.*

	-15-0 min	30-60 min	120-150 min
ERPF			
saline	3.4±0.4	3.0±0.2	3.3±0.5
propranolol	3.0±0.4	4.1±0.6	2.7±0.4
tertatolol low dose	3.1±0.6	4.0±0.3	2.0±0.2
tertatolol high dose	3.6±8.5	4.7±0.6*	2.7±0.5
GFR			
saline	0.90±0.10	0.90±0.07	0.92±0.07
propranolol	0.92±0.18	1.06±0.13	0.78±0.12
tertatolol low dose	0.69±0.11	1.02±0.15	0.58±0.08
tertatolol high dose	0.83±0.13	1.04±0.11	0.72±0.11
FF			
saline	0.26±0.01	0.31±0.03	0.29±0.02
propranolol	0.30±0.02	0.27±0.02	0.30±0.02
tertatolol low dose	0.24±0.03	0.25±0.02	0.28±0.01
tertatolol high dose	0.25±0.02	0.23±0.02	0.29±0.03
V			
saline	7.9±1.4	4.3±1.0	3.9±1.0
propranolol	5.4±1.3	9.6±1.8*	3.5±0.8
tertatolol low dose	7.8±1.8	12.0±1.5**	2.7±0.4
tertatolol high dose	6.9±2.7	13.0±2.4**	4.6±2.3

No significant differences were observed beween the pre-injection values for ERPF, GFR, FF and V in the 4 different experimental groups. Only 0.5 mg/kg tertatolol caused a significant increase in ERPF at 30 to 60 min after injection.

ERPF was at control levels 120 to 150 min post-injection in the 4 experimental groups. A slight but significant increase in GFR was observed after 30 to 60 min of administration of either beta-blocker. After 120 to 150 min, GFR had returned to control values. In the case of 0.1 mg/kg tertatolol, GFR decreased further to 0.58±0.08 ml/min.g kw but this reduction was not significantly different from control values. No statistically significant differences were observed

in urine excretion 15 to 0 min before the drug administration between the 4 experimental groups. Thirty to 60 min after 0.1 and 0.5 mg/kg tertatolol and 5 mg/kg propranolol, urine excretion increased significantly compared to control injections. The urine excretion returned to control values after 120 to 150 min.

6.3.4 Long-term effects on regional hemodynamics

Pre-infusion values of MAP (mm Hg) and HR (bpm) for the different experimental groups before the start of the infusion (day 0) are presented in table 6.4. No statistically significant differences were observed between pre-infusion values. Percentage change in MAP, HR, renal, hindquarter and mesenteric flow and resistance during chronic propranolol, tertatolol or saline infusion are presented in fig. 6.6. During long-term treatment with both beta-blockers propranolol ($p< 0.05$) and tertatolol ($p<0.001$), blood pressure was significantly reduced to new steady-state levels 3 days after the start of the infusion. Blood pressure was reduced more during infusion of tertatolol

Table 6.4: Pre-infusion values (means ± SEM) of MAP (mm Hg) and HR (bpm) of the saline, 5 mg/kg.d propranolol and 0.5 mg/kg.d tertatolol treated group in the long-term regional hemodynamic study.

	n	MAP	HR
0.9% NaCl	13	155±3	315±11
Propranolol	7	148±4	339±7
Tertatolol	7	169±4	332±7

than during propranolol infusion (-28±1% and -12±1%, respectively). Both beta-blockers caused a fall (-8±2%; $p<0.05$, and -13±3%; $p<0.001$ respectively) in HR. This effect was significant from the second day and did not change on subsequent days.

Propranolol reduced renal blood flow significantly ($p<0.05$) to

a new steady-state level during the first infusion day. No change in renal blood flow was observed in the case of tertatolol. Both beta-blockers similarly reduced hindquarter flow during the first 2-3 days of infusion to a maximum of -31±6% ($p<0.001$) in the case of propranolol and -28±3% ($p<0.001$) in the case of tertatolol. Thereafter, for both drugs a little increase in hindquarter flow was observed.

Also the effect on MF was nearly equal for both beta-blockers. MF was reduced during the first two infusion days. This significant reduction was similar during the following infusion days (maximally

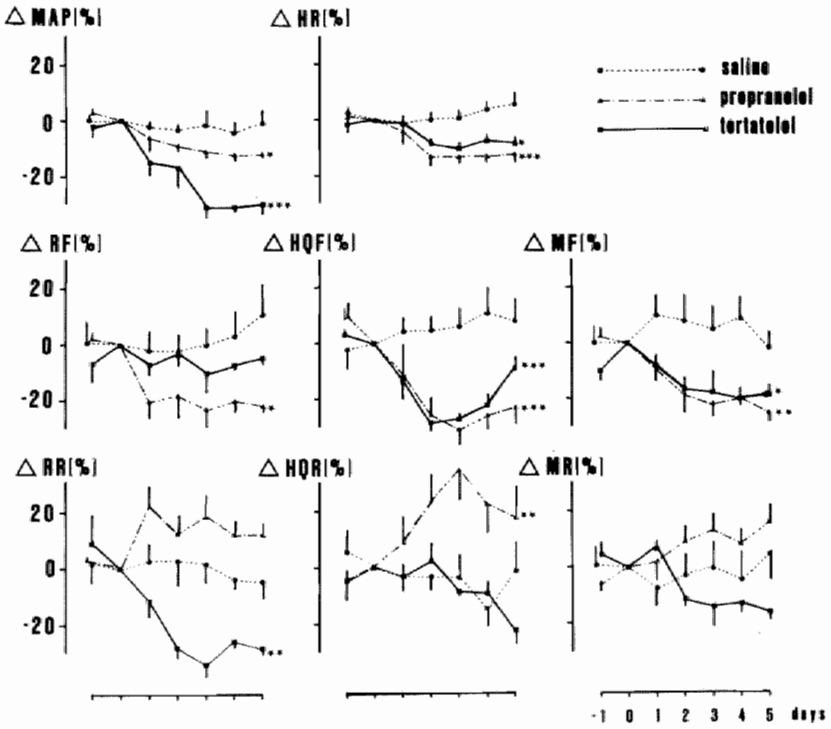


*Fig. 6.6: Effects of long-term infusion of saline (24 µl/d), propranolol (5 mg/kg.d) and tertatolol (0.5 mg/kg.d) on mean arterial pressure (MAP), heart rate (HR), renal flow (RF), hindquarter flow (HQF), mesenteric flow (MF), renal resistance (RR), hindquarter resistance (HQR) and mesenteric resistance (MR) in conscious SHR. Significances are given in comparison with the saline infusion: *p<0.05; **p<0.01; ***p<0.001.*

-20±4%, $p<0.05$ and -25±3%, $p<0.01$ for tertatolol and propranolol respectively).

Regional resistance changes were calculated from pressure and flow changes. Tertatolol significantly ($p<0.01$) reduced RR but did not influence HQR and slightly but not significantly reduced MR. Propranolol increased renal, hindquarter and mesenteric resistance but only the effect on HQR was significantly different from the control values ($p<0.01$). The slight reduction in MR during chronic tertatolol treatment was significantly different from the slight increase in MR in the case of propranolol ($p<0.001$).

6.3.5 Long-term effect on plasma renin concentration

The values of MAP, HR and PRC just before the start and after 4 days of infusion of 5 mg/kg.d propranolol, 0.5 mg/kg.d tertatolol and saline are summarized in table 6.5.

Long-term infusion of propranolol and tertatolol significantly reduced MAP and HR. PRC was significantly reduced (37%, $p<0.05$) in the case of propranolol, but not during tertatolol.

*Table 6.5: Blood pressure (MAP, mm Hg), heart rate (HR, bpm) and plasma renin activity (PRC, ng AI/ml.hr) in the different experimental groups just before (day 0) and after a 4-day infusion period of the respective drugs. Data are expressed as mean ± SEM. Significances are given in comparison to pre-infusion values: *p<0.05; **p<0.01; ***p<0.001.*

	n	Day	MAP	HR	PRC
0.9% NaCl (0.1 ml)	6	0	167±7	325±7	7.1±0.8
		4	168±7	338±9	7.5±1.1
Propranolol (5 mg/kg.d)	11	0	170±4	328±10	8.4±0.8
		4	137±4***	297±5*	5.3±0.5*
Tertatolol (0.5 mg/kg.d)	7	0	174±3	358±6	8.1±1.2
		4	139±5***	304±13**	7.5±1.3

6.4 Discussion

In this study we compared the hemodynamic actions of the non-selective betablockers propranolol and tertatolol in conscious unrestrained SHR, instrumented for continuous measurements of central or regional hemodynamic variables.

The choice of dosages for the two drugs was based upon a study by Laubie et al (1973). In that study similar beta-adrenoceptor blockade was observed for propranolol in dogs at doses of a factor ten-twenty higher than for tertatolol. Because in most in vivo studies in rats (Davy et al, 1977; Smits et al, 1980a,b, 1982; Struyker-Boudier et al, 1979) 5 mg/kg propranolol was used as a maximal i.v. doses, in our studies a similar dosage was used for propranolol and 0.5 mg/kg was used as a maximal dosage for tertatolol.

Our data confirm previous reports on the time dependent effects of beta-adrenoceptor blockers (Smits et al, 1979, 1982). Thus, an immediate reduction of cardiac output was observed whereas the change in MAP consisted of an early rise, followed by a later decrease. The early rise in MAP was more pronounced in the case of tertatolol compared to the early effect of propranolol on MAP, reported by Smits et al (1982). Previous studies (Struyker-Boudier et al, 1979; Smits et al, 1982) showed that the hemodynamic action of propranolol consist of an acute as well as of a long-term reduction in cardiac output, whereas the lack of acute decrease in blood pressure is caused by a baroreflex mediated rise in total peripheral resistance (TPR). This hemodynamic pattern suggests that the fall in cardiac output rather than blood pressure triggers a baroreflex response. Experiments by Charlton and Baertschi (1982) indicate that in the rat a change in aorta blood flow can indeed affect baroreflex activity. Cardiac output decreased to a similar extent in the case of 0.5 mg/kg tertatolol in our study as compared to that of 5 mg/kg propranolol described by Smits et al (1982). If we assume that the reduction in cardiac output is responsible for the increase in TPR we would expect a slower increase in TPR in the case of tertatolol as compared to propranolol. However, this was not found in our study. We observed a

similarly fast increase in TPR after tertatolol as was reported for propranolol. These results suggest that tertatolol may have a direct vasoconstricting or baroreflex stimulating activity, or propranolol may have a direct vasodilating or baroreflex reducing activity at several not measured vascular beds.

We furthermore investigated the possible differences in regional sensitivity of different vascular beds in the vasoconstrictor effects of beta blockers. Other authors, using the microsphere technique in several animal models, showed that propranolol causes an early generalized increase in vascular resistance in a number of vascular beds (Nies et al, 1973; Van Boom and Saxena, 1983; Hatzinikolaou et al, 1983). Also in clinical experiments, a rise in vascular resistance was observed in different vascular beds, following the acute administration of propranolol (cf. Fitzgerald, 1984; Van Baak et al, 1985). Again, our data confirm these observations for propranolol. Since this drug caused a comparable increase in vascular resistance in the renal, hindquarter and mesenteric beds. Tertatolol clearly differs from propranolol with respect to its regional hemodynamic effects. Tertatolol caused an early increase only in hindquarter resistance. Rise in renal and mesenteric resistances were not observed and 3-4 hr after bolus injection of tertatolol renal resistance was even decreased. From the central hemodynamic studies we would expect a more pronounced increase in regional resistances in the case of tertatolol as compared to propranolol. However, the reverse was observed in the regional hemodynamic study. This suggest that tertatolol causes a vasoconstriction in other vascular beds than were studied in our regional hemodynamic study.

We have investigated the role of the baroreflex in these regional hemodynamic effects of both betablockers in conscious SHR. For this purpose we determined these effects in intact and baroreceptor denervated animals. After removal of the sino-aortic baroreflex control, propranolol and tertatolol behaved similarly. A reduction in renal, an increase in hindquarter and no effect on mesenteric resistance was observed for both beta-blockers in denervated rats. These results suggest that tertatolol reduces the baroreflex mediated constriction in the renal and mesenteric vascular beds in intact animals.

Furthermore, these results indicate that tertatolol may accentuate a baroreflex mediated vasoconstriction in other vascular beds with an exception for the hindquarter. In the muscular vascular bed a role of the baroreflex can be excluded.

The question can be raised trough what mechanisms tertatolol interferes with the baroreflex. Verbeuren et al (1985) showed that tertatolol has a high affinity for prejunctional beta-adrenoceptors in sympathetic nerve terminals in the dog saphenous vein. Thus it blocked the increased stimulation evoked overflow of norepinephrine induced by isoprenaline. So it seems attractive to speculate that the lack of vasoconstrictor activity of tertatolol in some vascular beds is due to its prejunctional effect. However, Laubie et al (1986) reported that pressor responses to a number of constrictor agents ($BaCl_2$, serotonin) are inhibited by tertatolol, but not by propranolol or nadolol. This would suggest a non-adrenoceptor effect of the drug in the renal and mesenteric vasculature.

A alternative hypothesis for the mechanism by which tertatolol influences the baroreflex mediated effects could be that it interferes with central sites involved in the baroreflex pathway. Such an interference was suggested for atenolol by Scott (1983) and Scott and Williams (1982) in anesthetized cats. Interaction studies of tertatolol with sites in the central nervous system have not yet been reported.

We have shown above that the baroreflex plays an important role in the acute regional hemodynamic effects of both beta-adrenoceptor blockers. On the long-term the influence of baroreceptor reflex mechanisms may be less dominant, because of adaptation of these mechanisms to the prevailing level of blood pressure. Therefore we extended our study on regional hemodynamics to their chronic application.

During long-term treatment, propranolol induced slight increases in renal and mesenteric resistances and a pronounced increase in hindquarter resistance. In the case of tertatolol hardly any changes in hindquarter and mesenteric resistance were observed but renal resistance decreased significantly during long-term treatment. The long-term changes are almost similar to the acute regional hemodynamic

changes, measured in sino-aortic baroreceptor denervated animals in the case of tertatolol. This in contrast to propranolol whose long-term hemodynamic effects were better comparable with the acute regional hemodynamic effects in the intact animals. These results suggest a more rapid baroreceptor resetting during long-term tertatolol treatment as compared to propranolol. Possibly this difference in baroreflex resetting for both beta blockers is based on their difference in renal perfusion. In the case of propranolol an acute and long-term reduction in renal blood flow was observed. This in contrast to tertatolol that did not change renal blood flow. Moss (1986) has reported that a reduction in renal perfusion induces an excitation of renal afferent nerves. Furthermore, Janssen et al (1986) have shown that interruption of renal afferent nerves changes the baroreflex sensitivity. So it cannot be excluded that long-term differences in renal perfusion may leed to differences in baroreceptor resetting. This has to be further investigated.

In the acute situation and during chronic tertatolol treatment renal blood flow was left intact. This in contrast to the reduction in renal blood flow seen in the case of propranolol. This effect of tertatolol on renal vasculature may have important therapeutic implications since it may be expected to promote excretion of water and sodium (Borst and Borst-de Geus, 1963; Guyton et al, 1979). Therefore we extended our study with acute renal hemodynamic and excretory function measurements in conscious SHR.

In these studies we found that in contrast to the significant reduction in renal blood flow in the regional hemodynamic study the effective renal plasma flow (ERPF) was not affected after propranolol in the renal hemodynamic study. In the case of tertatolol no change in renal blood flow but an increase in ERPF was observed. The clearance of para-aminohippurate (PAH) was used to determine ERPF. Because PAH is excreted in the cortical part of the kidney. So the differences seen between renal blood flow and ERPF after beta blockade indicate an intrarenal redistribution of flow to the cortex of the kidney possibly as a consequence of a medullary vasoconstriction induced by beta-adrenoceptor blockade. De Leeuw et al (1982) observed a better cortical renal blood flow in hypertensive patients treated with propranolol as

compared to non-treated hypertensive subjects. These observations support our suggestion for the difference seen between renal blood flow and ERPF after beta-blockade.

No differences in GFR were observed as compared to control values for both beta-blockers. Furthermore both drugs induced a considerable diuresis. These results suggest a beta-adrenoceptor mediated reduction in tubular reabsorption. These observations differ from previously published results by Laubie et al (1986). They found that in contrast to propranolol, tertatolol increased GFR and urine and sodium excretion in conscious dogs. In these studies they observed no effect of both beta blockers on ERPF. In another study the same investigators (Lantz et al, 1984) observed increases in ERPF and GFR in hypertensive patients given tertatolol orally. Possibly these different experimental results can be explained by species differences because a similar effect of propranolol on urine excretion was observed by Smits et al (1982) in conscious SHR. He suggested an involvement of the sympathetic nervous system in the diuretic and natriuretic effects of propranolol. This hypothesis was supported by other investigators. Bencsath et al (1979) showed that renal sympathectomy increased sodium and water excretion in rats. Schrier (1972), Besarab et al (1977) and Bello-Reus (1980) showed that beta adrenoceptors are involved in the increase of tubular reabsorption of water and sodium during renal nerve stimulation. These authors suggest that renal sympathetic nervous activity has an inhibitory effect on water and sodium excretion through beta-adrenergic mechanisms and consequently blockade of beta- adrenoceptors may lead to a diuresis and natriuresis by reducing tubular reabsorption. Struyker Boudier et al (1986) reported that high amounts of beta-adrenoceptor binding sites are present in the rat kidney tubulus. These observations support the above hypothesis.

Several investigators, however, showed that the increased tubular reabsorption of water and sodium is mediated through alpha-adrenergic mechanisms (DiBona, 1977; DiBona et al, 1977; Colindres et al, 1978).

Besides an influence via beta-adrenoceptors in the kidney also hormonal changes may be involved in these tubular effects. A possible

candidate could be the renin-angiotensin-aldosteron system. A reduction in plasma renin concentration (PRC) and consequently a decrease in angiotensin II and aldosteron might provide an alternative explanation for the diuresis seen after beta-blockade. Beta-adrenoceptor blocking agents reduce PRC in rats (Bühler et al, 1972; Leenen and Ackermann, 1976; Fernandes et al, 1976, 1977; Niarchos et al, 1977; Hopak et al, 1977; Caputi et al, 1978; Gulati and Liard, 1979, Smits, 1980b). In our study we measured plasma renin concentration after a four days infusion period of both beta blockers. We observed a reduction in PRC in the case of propranolol. However, no change in PRC was observed during long-term tertatolol treatment. It is not unlikely that in the case of propranolol the renin angiotensin aldosteron system is involved in the observed tubular effects.

In conclusion, our data show that activation of the sino-aortic baroreflex plays an important role in the early and long-term hemodynamic effects of both beta blockers propranolol and tertatolol. Furthermore, the data suggest that the absence of an increase in renal and mesenteric resistance following tertatolol is related to an interaction of the drug with the sympathetic nervous system mediated reflex vasoconstriction. Tertatolol thus protects the kidney from a renal hypoperfusion as was observed for propranolol. This could have a potentially therapeutic advantage in the treatment of hypertension.

CHAPTER 7

HEMODYNAMIC EFFECTS OF THE RENAL VASODILATOR PRODRUG CGP 22 979A AND ITS PARENT COMPOUND CGP 18 137A IN CONSCIOUS SHR

7.1 Introduction

Vasodilator drugs with a selective or preferential renal action (see chapter 1) have been suggested to have a potential usefulness in disease states such as hypertension and renal failure (Struyker Boudier, 1980; Ackermann et al, 1982, 1983) although others have challenged this suggestion (Brenner et al, 1982). Selective renal vasodilation is described for converting enzym inhibitors (Richer, 1983; Oliver et al, 1983; Smits et al, 1984) and for several naturally occurring prostaglandins. Also beta-blockers which maintain normal renal perfusion like tertatolol (Lanz et al, 1984; chapter 6 of the thesis) and nadolol (Danesh et al, 1984) may be mentioned in this respect. Furthermore, substances were developed like (+)-4-3-3-[2-(1-hydroxycyclohexyl)-ethyl]-4-oxo-2-thiazolidinyl propyl benzoic acid, which dilates the renal vasculature through interference with prostaglandin mechanisms (Blaine et al, 1982; Seymour and Blaine, 1983), and fenoldopam (SK&F 82526), which is thought to cause renal vasodilation through stimulation of postsynaptic dopamine D_1 receptors in the kidney (Ackermann et al, 1982, 1983). Another possible approach was introduced by Hofbauer et al (1985), i.e. the use of the prodrug principle to achieve selectively or preferentially high concentrations of a vasodilator substance in the kidney. They described the pharmacology of the prodrug CGP 22 979A in anesthetized rats and compared it to that of its parent substance CGP 18 137A. Thus, in CGP 22 979A, an N-acetyl-L-gamma-glutamyl moiety is added to CGP 18 137A, which is to be regarded as a hydralazine-like vasodilator. Sequential hydrolysis by acylase and glutamyltranspeptidase is needed in order to generate the active substance. These reactions occur in the kidney at a higher

rate than in other tissues (Orlowski et al, 1980). Addition of an N-acetyl-L-gamma-glutamyl moiety to the model substance sulfamethoxazole has, for instance, been shown to result in a highly selective accumulation of the active compound in mouse kidneys (Orlowski et al, 1980).

The first purpose of the present study was to quantitate the acute regional hemodynamic effects of CGP 22 979A to investigate its renal specificity. These effects were compared with the regional hemodynamics of its active compound CGP 18 137A.

Computer simulation studies suggest (see chapter 1) that selective renal vasodilation leads to an acute diuresis and natriuresis decreasing blood volume and, consequently, cardiac output. Such a diuresis was experimentally confirmed for CGP 22 979A by Hofbauer et al (1985) in anesthetized rats. We extended our studies to investigate central and renal hemodynamics and renal excretory functions after bolus injections of CGP 22 979A and CGP 18 137A in conscious SHR.

Furthermore, computer simulation studies of selective renal vasodilators suggest a more pronounced reduction in blood pressure during long-term treatment induced by a slow reduction in TPR without a change in plasma angiotensin II levels. Therefore, we determined in addition the regional hemodynamic effects and the effect on plasma renin concentration of the possible preferential renal vasodilator CGP 22 979A and the hydralazine-like vasodilator CGP 18 137A during a continuous administration period of 5 days in conscious SHR.

7.2 Experimental protocol

7.2.1 Animals

Male SHR, weighing 250-350 g, were used in the studies described in this chapter. More details are presented in section 2.1 of this thesis.

7.2.2 Central hemodynamic studies

SHR used for these studies were instrumented as described in paragraph 2.3. On the experimental day, the animals were placed in 30x20x30 cm experimental cages. The arterial catheter was connected to

a pressure transducer (CP-01; CTC, Inglewood, Ca, USA) to record blood pressure. MAP was obtained by low-pass filtering. The electromagnetic flowprobe was connected to a sine-wave flowmeter (MDL 400, Skalar, Delft, The Netherlands). The flat part of late diastolic flow was taken to be zero. The mean aortic flow that was obtained by low-pass filtering and which in fact constitutes CO minus coronary flow, will be referred to furtheron as CO. HR was determined from the pulsatile flow signal using a biotacho-coupler (Narco, Houston, TX, USA). All signals were monitored on a Grass model 7D polygraph (Grass Instruments, Quincy, MA, USA).

CI was calculated as CO/100 g bw. SVI was derived from CI and HR. TPRI was calculated as MAP/CI.

Injections were given after animals had been connected to the equipment for at least 1 hr. After determination of base-line values as the means of 4 consecutive readings 5 min apart, i.v. injections of saline (0.1 ml), CGP 18 137A (0.3-1 mg/kg) or CGP 22 979A (3-30 mg/kg) were given and hemodynamics were monitored for at least 4 hr. Values stated for hemodynamic parameters at 0.5 to 1 and 3 to 4 hr are averages from 5 and 10 readings during these periods, respectively.

7.2.3 Regional hemodynamic studies

Surgery, measurements and calculations concerning the regional hemodynamics were performed as described in paragraph 2.4. After connecting the head plug and catheters, the animals were allowed 1 to 2 hr to adjust to the experimental conditions. Arterial blood pressure was measured with a miniature pressure transducer (CP-01; CTC, Inglewood, CA, USA) connected to the arterial catheter. MAP was obtained by electronic damping. HR was derived from the pressure signal using a Narco biotacho-coupler (Narco Bio-Systems, Houston, TX, USA). The Doppler probes were connected to a 4-channel 20 MHz directional pulsed Doppler system (Bioengineering Department, University of Iowa, Iowa City IA, USA).

Injections were given in 0.1 ml of 0.9% NaCl and catheters were flushed slowly with 0.2 ml of 0.9% NaCl. After the injection, signals were monitored continuously for a period of 2 to 3 hr. Different dosages of the drug were given on different days in random order.

Seven animals received 0.3 ml of 0.9% NaCl after which parameters were monitored for 1 hr. These animals served as controls.

In the long-term studies, surgery was extended with an implantation of a jugular vein catheter (see paragraph 2.2.3). Regional hemodynamics were measured every day (during ca. 1.5 hr), one day before to 5 days after the start of the infusion of 1 mg/kg.d CGP 18 137A, 10 mg/kg.d CGP 22 979A or saline. In these studies, doses of CGP 22 979A and CGP 18 137A were used as described in a preliminary study (Hofbauer et al, 1986). After the measurements on day 0, the infusion was started. For this purpose the drug containing Alzet^{TM} minipump was connected to the jugular vein catheter and implanted under the skin of the lightly ether anesthetized rat. The effects are expressed as percentage change from pre-infusion values measured on day 0.

7.2.4 Renal hemodynamic studies

Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured in conscious SHR as plasma clearances of [^{51}Cr]-EDTA and [^{125}I]PAH, respectively, as described in paragraph 2.8 "Method A". During experiments, rats were in their own cages. Tracers were injected immediately after CGP 18 137A or CGP 22 979A or 3 hr after administration of the drugs. Thus, values of GFR and ERPF were obtained for 0 to 1.5 hr and 3 to 4.5 hr after injections. Control animals received 0.3 ml of 0.9% NaCl immediately before tracers. At the end of the experiments, kidneys were removed, blotted and weighed. GFR and ERPF are expressed as milliliters per minute per gram of kidney weight. The filtration fraction (FF) was calculated as GFR/ERPF.

7.2.5 Renal function studies

Urine (V) and sodium excretion ($U_{Na}V$) were quantitated in conscious SHR as described in paragraph 2.9 of this thesis. On the experimental day, the animals were placed in metabolic cages and sodium and water intake was controlled. The outlet of the metabolic cages was connected to an LKB model 700 fraction collector which was set to change tubes every 120 min. V was determined every 2 hr by weighing the tubes. Na^+ concentration was measured using a flame photometer.

After 12 to 20 hr, bolus injections of CGP 18 137A (1 mg/kg) or CGP 22 979A (3-30 mg/kg) were given and V and $U_{Na}V$ were monitored for three more 2 hr periods.

7.2.6 Plasma renin concentration studies

Plasma renin concentration (PRC) was quantified by measuring the rate of angiotensin I (AI) formation by renin in plasma incubated under optimal conditions for renin (see paragraph 2.7).

The SHR used for these studies were implanted with an abdominal aorta and a jugular vein catheter (see paragraph 2.2.1 and 2.2.3). The arterial catheter was used for MAP and HR measurements and blood sample collection. The venous catheter was used for long-term infusions of CGP 18 137A (1 mg/kg.d), CGP 22 979A (10 mg/kg.d) or saline using Alzet^{TM} osmotic minipumps. Similar doses were used as in the long-term regional hemodynamic studies. MAP and HR were measured every day (ca. 1.5 hr) one day before to 5 days after the start of the infusion.

Blood samples (250 µl) were taken just before and after 4 days of infusion. Blood samples were centrifugated at 2000 rpm and the plasma was stored at -80°C until plasma renin activity was determined.

7.3 Results

7.3.1 Effects on central hemodynamics

Base-line values for the experimental groups used in central hemodynamic studies are summarized in table 7.1. There were no significant differences between starting values in the different groups. Percentages of changes in hemodynamic parameters after saline, CGP 18 137A and CGP 22 979A are shown in fig. 7.1. Saline induced only minor changes at 0.5-1 and 3-4 hr after injection. CGP 18 137A caused a rapid dose-dependent reduction of MAP (cf. fig. 7.1). This effect was associated with a steep fall in TPRI and increased CI because of a pronounced tachycardia and a slightly decreased SVI. At 3 to 4 hr after injections, all effects were smaller than at 0.5 to 1 hr after injections (cf. fig. 7.1) although decrease in MAP and TPRI and in-

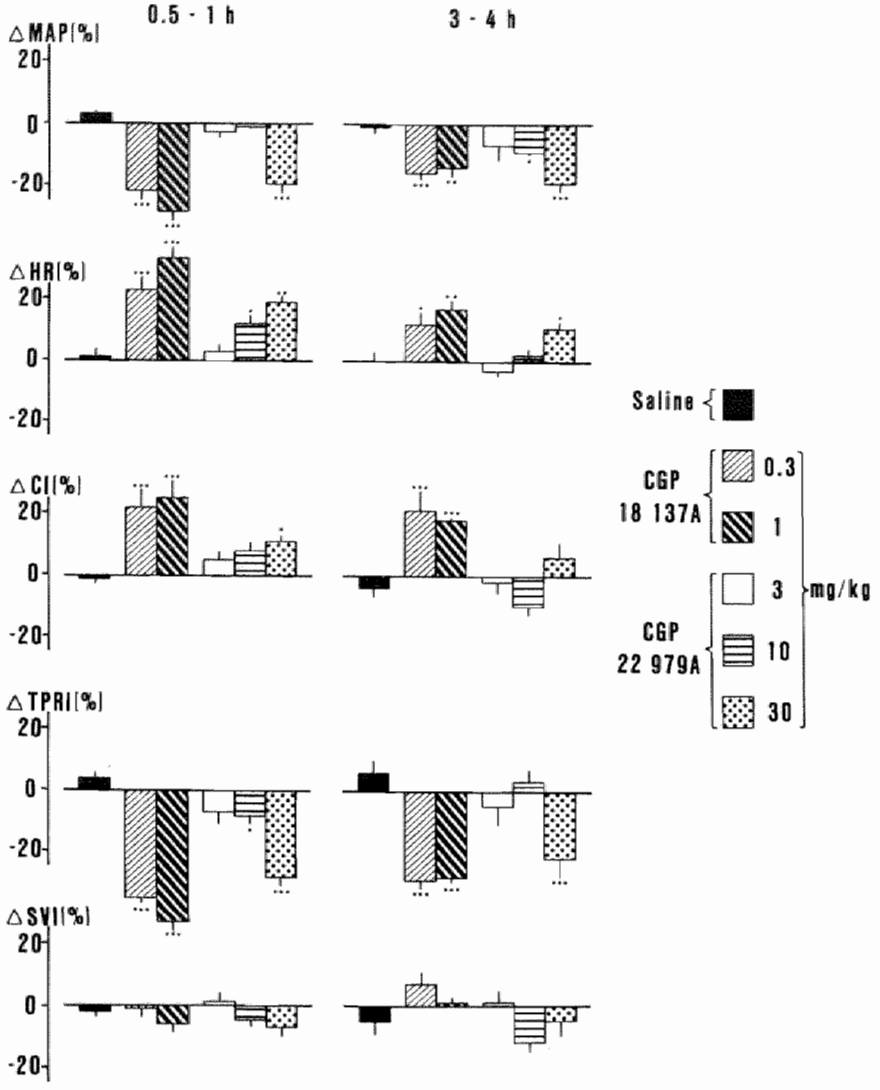


*Fig. 7.1: Effects (percentage changes) of saline, CGP 18 137A and CGP 22 979A on MAP, HR, CI, TPRI and SVI at 0.5 to 1 hr respectively 3 to 4 hr after injections. Significances in differences with saline: *p<0.05; **p<0.01; ***p<0.001.*

Table 7.1: Pre-injection values of MAP, HR, CI, TPRI and SVI in the experimental groups used for central hemodynamic studies.

Treatment	n	MAP mm Hg	HR bpm	CI ml/min. 100 g bw	TPRI mm Hg/min. 100 g bw. ml	SVI µl/100 g bw
Saline	10	139± 3	395±14	31±3	4.8±0.4	79±7
CGP 18 137A						
0.3 mg/kg	7	130±4	384±17	34±1	3.9±0.2	88±3
1 mg/kg	7	127±10	372±20	31±1	4.2±0.5	87±7
CGP 22 979A						
3 mg/kg	6	142±7	373±6	31±3	4.8±0.6	84±8
10 mg/kg	9	153±5	398±9	32±1	4.6±0.3	81±4
30 mg/kg	6	133±4	396±14	30±2	4.5±0.3	77±6

creases in HR and CI were still significant.

The two lowest doses of CGP 22 979A (3 and 10 mg/kg) did not cause immediate changes in MAP. TPRI decreased slightly but significantly after 10 mg/kg ($p<0.05$). However, this reduction (−8±3%) was small as compared to the changes at 0.5 to 1 hr after even the lowest dose of CGP 18 137A (−35±2%). HR increased (+9±3%; $p<0.05$) after 10 mg/kg, but CI and SVI were not significantly affected (cf. fig. 7.1). At this time, the higher dose of CGP 22 979A (30 mg/kg) did reduce MAP (−20 ±3%; $p<0.001$). TPRI was reduced (−28±3%; $p<0.001$). HR (+19±2%; $p<0.01$) and CI (+11±2%; $p<0.05$) increased only slightly, whereas SVI tended to decrease.

At 3 to 4 hr after injections of all three doses of CGP 22 979A, MAP was decreased (cf. fig. 7.1). Only in the group receiving 30 mg/kg, a significant reduction in TPRI was noted. In the group injected with 10 mg/kg, CI tended to fall (−10±3%) whereas in the 30 mg/kg group, it was not changed (+6±5%). SVI was insignificantly affected by −12±3% and −5±5%, respectively in the 10 and the 30 mg/kg groups.

7.3.2 Acute effects on regional hemodynamics

Pre-injection absolute values for MAP and HR are summarized in

Table 7.2: Pre-injection absolute values of MAP and HR in the different experimental groups used in the regional hemodynamic studies.

	Dose (mg/kg)	n	MAP (mm Hg)	HR (bpm)
Saline		7	152±17	350±24
CGP 18 137A	0.1	5	140±18	366±29
	0.3	7	136± 9	383±54
	1.0	7	149±11	322±33
CGP 22 979A	1	5	159±25	308±45
	3	8	151±12	325±34
	10	8	154±18	331±44
	30	8	151±13	338±26

table 7.2. Saline caused only minor changes in MAP, HR and the regional resistances. CGP 18 137A was injected in doses of 0.1 (n=5), 0.3 (n=7) and 1.0 mg/kg (n=7). The time course of the reduction of MAP and increases in HR was comparable to those seen after hydralazine (see chapter 4). Also, with regard to its antihypertensive potency, CGP 18 137A (fig. 7.2) was fully comparable to hydralazine (see fig. 4.4). A maximal reduction of MAP by 41±7 mm Hg was observed after 1 mg/kg i.v. The effect of CGP 18 137A on HR tended to be greater than that after hydralazine. All three vascular resistances decreased significantly at all doses of CGP 18 137A tested (cf. fig. 7.2). Although the effects on MR were comparable to those of hydralazine, the effect of CGP 18 137A on HQR was identical to that of hydralazine only at the two lowest doses, whereas 1 mg/kg of CGP 18 137A decreased HQR less. The reductions of RR, although significant at all doses, were smaller after CGP 18 137A (fig. 7.2) than after hydralazine (fig. 4.4). Thus, the reduction of RR following hydralazine and CGP 18 137A never exceeded that of HQR and MR.

CGP 22 979A was injected in doses of 1 (n=5), 3 (n=8), 10 (n=8) and 30 mg/kg (n=8). Significant changes of MAP and HR were only seen after the two highest doses. These effects developed more gradually

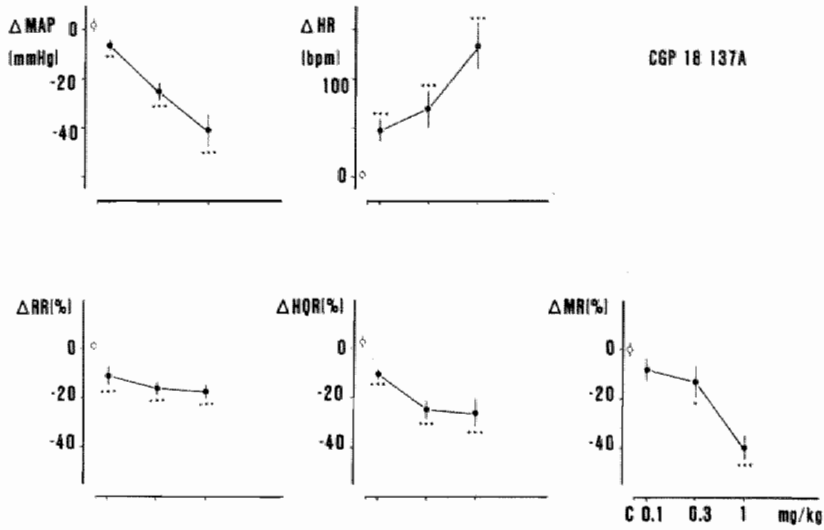


*Fig. 7.2: Effects of CGP 18 137A on regional hemodynamics in conscious SHR. Significances are given in comparison to saline controls (C): *p<0.05; **p<0.01; ***p<0.001.*

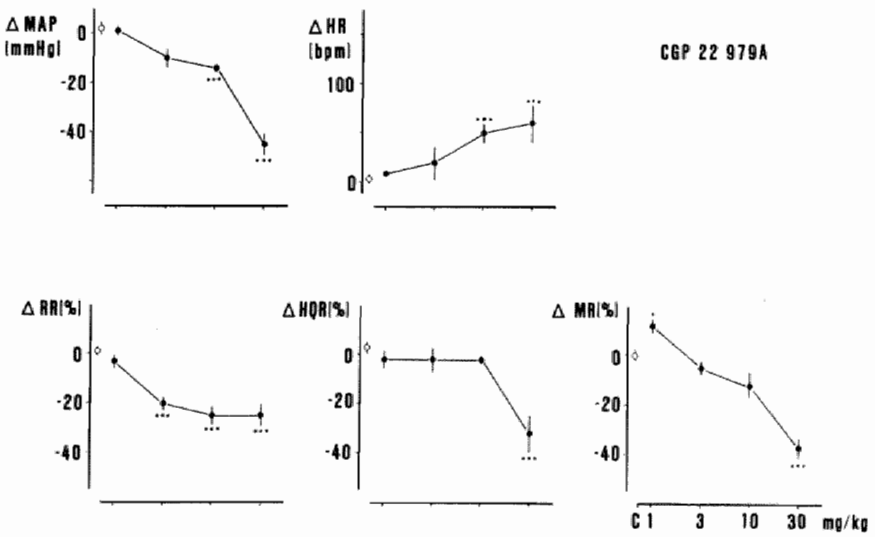


*Fig. 7.3: Effects of saline and CGP 22 979A on regional hemodynamics in conscious SHR. Significances are given in comparison to saline controls (C): *p<0.05; **p<0.01; ***p<0.001.*

than those after hydralazine or CGP 18 137A. A maximum decrease in blood pressure was observed after 45 to 60 min. The effects of CGP 22 979A are summarized in fig. 7.3. The magnitude of the effect of 30 mg/kg of CGP 22 979A on MAP (-44±5 mm Hg) was comparable to that of 1 mg/kg CGP 18 137A (-41±7 mm Hg), making it much less potent as an acutely blood pressure lowering agent. HR increased less with CGP 22 979A than with the parent compound. Again, after the highest dose, it increased by 59±19 beats/min, which is significantly less ($p<0.05$) than the increase of 134±27 beats/min seen after 1 mg/kg of CGP 18 137A.

HQR did not change significantly after 1 to 10 mg/kg of CGP 22 979A, whereas MR increased significantly after 1 mg/kg and was not significantly changed after 3 to 10 mg/kg of CGP 22 979A. Only after the highest dose of 30 mg/kg of CGP 22 979A a significant decrease in HQR and MR was observed. In contrast, RR decreased significantly at all doses but the lowest. The maximal effect on RR was already seen after 10 mg/kg (-25±4%) after which dose neither HQR (-2±2%) nor MR (-12±5%) were decreased significantly. Thus, after 3 and 10 mg/kg of CGP 22 979A, RR decreased to a greater extent than HQR and MR. After 30 mg/kg, a comparable degree of resistance reduction was observed in the three vascular beds.

7.3.3 Effects on renal hemodynamics

The valules of GFR, ERPF and FF measured 0 to 1.5 and 3 to 4.5 hr after bolus injections of saline, CGP 18 137A (1 mg/kg) and CGP 22 979A (3-30 mg/kg) are presented in fig. 7.4. In saline-injected animals, ERPF was 3.70±0.26 ml/min.g kw. One mg/kg of CGP 18 137A decreased ERPF to 2.85±0.49 ml/min.g kw. at 0 to 1.5 hr, although this value was not significantly different from the value after saline. At 3 to 4.5 hr, it was back to 3.41±0.42 ml/min.g kw. CGP 22 979A increased ERPF dose-dependently at 0 to 1.5 hr after injection. After 30 mg/kg, ERPF was 4.90±0.17 ml/min.g kw. ($n=6$; $p<0.05$). At 3 to 4.5 hr after injections of CGP 22 979A, ERPF was still slightly increased (4.38±0.29 ml/min.g kw. after 30 mg/kg).

GFR was 1.17±0.12 ml/min.g kw ($n=7$) in saline-injected animals. CGP 18 137A caused a rapid, significant ($p<0.001$) reduction of GFR at

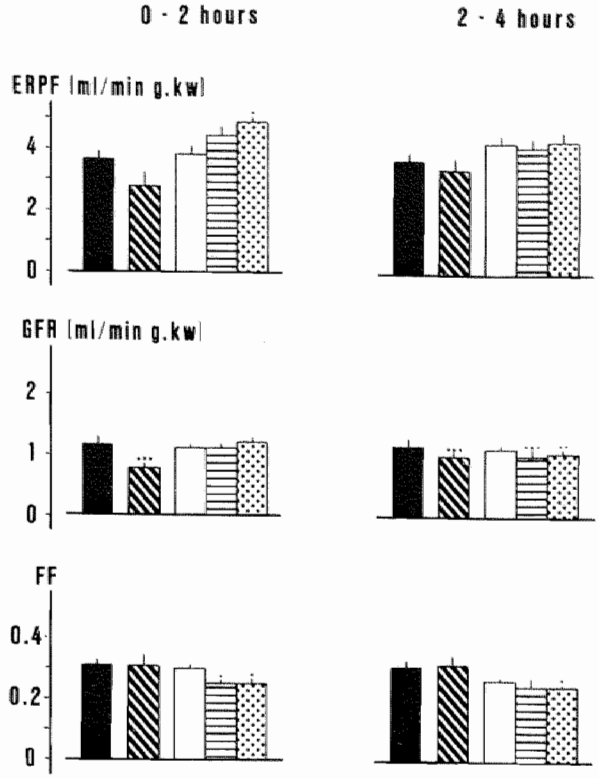


*Fig. 7.4: Effects of saline, CGP 18 137A and CGP 22 979A on ERPF, GFR and FF at 0 to 1.5 hr respectively 3 to 4.5 hr after injection. Significances in differences with saline: *p<0.05. For symbols, see legend to fig. 7.1.*

0 to 1.5 hr after injection (0.79±0.06 ml/min.g kw.; n=6). At 3 to 4.5 hr after injection, it was still reduced. CGP 22 979A did not affect GFR at 0 to 1.5 hr after injection. At 3 to 4.5 hr after injection, both 10 and 30 mg/kg reduced GFR slightly, but significantly ($p<0.01$; cf. fig. 7.4).

After saline, FF was 0.31±0.02. CGP 18 137A did not affect FF (cf. fig. 7.4). However, all doses of CGP 22 979A decreased FF. After 30 mg/kg it decreased to 0.25±0.02 ($p<0.05$) and this effect persisted at 3 to 4.5 hr after injection (0.25±0.01; $p<0.05$).

*Table 7.3: Effects (means ± SEM) of aline, CGP 18 137A and CGP 22 979A on diuresis and natriuresis in conscious SHR. Significances in differences with saline: *p<0.05.*

	n	hr post injection				
		-4 to -2	-2 to 0	0 to 2	2 to 4	4 to 6
V (ml/2hr)						
Saline	8	0.81±0.21	0.80±0.27	1.02±0.18	0.84±0.19	0.90±0.37
CGP 18 137A						
1 mg/kg	7	0.82±0.12	1.03±0.14	0.69±0.25	0.58±0.29	1.53±0.34
CGP 22 979A						
1 mg/kg	7	0.88±0.23	0.73±0.25	2.14±0.57	1.13±0.35	1.20±1.26
3 mg/kg	6	0.86±0.27	0.87±0.26	3.15±0.79	1.42±0.24	0.76±0.51
10 mg/kg	7	0.85±0.16	0.72±0.19	3.33±0.58*	0.96±0.39	0.42±0.16
30 mg/kg	7	0.67±0.09	0.71±0.07	3.48±0.61*	0.41±0.25	1.87±0.44
$U_{Na}V$ (µEq/2hr)						
Saline	8	128 ±34	131 ±48	142 ±44	120 ±26	150 ±46
CGP 18 137A						
1 mg/kg	7	213 ±53	189 ±45	144 ±62	114 ±86	430 ±164
CGP 22 979A						
1 mg/kg	7	178 ±33	153 ±33	538 ±195	230 ±91	351 ±92
3 mg/kg	6	168 ±42	168 ±39	492 ±149	200 ±42	149 ±73
10 mg/kg	7	170 ±49	200 ±56	483 ±133	283 ±184	71 ±35
30 mg/kg	7	112 ±29	122 ±32	509 ±148*	78 ±62	288 ±92

7.3.4 Effects on renal excretory function

Effects of saline, CGP 18 137A and CGP 22 979A on diuresis and natriuresis are shown in table 7.3. Base-line values for diuresis were between 0.67 and 1.03 ml/2 hr, whereas sodium excretion ranged from 128 to 213 µEq/2 hr. There were no significant differences between base-line values for the groups. Saline caused only marginal changes in water and sodium excretion. CGP 18 137A reduced V and $U_{Na}V$ during the first two periods, whereas it slightly increased them in the last 2 hr period. Changes were insignificant as compared to controls. CGP 22 979A caused a dose-dependent increase in diuresis during the first

2 hr period (cf. table 7.3). Also $U_{Na}V$ increased although there was no relationship between dose and magnitude of the increase. After this initial increase, V and $U_{Na}V$ decreased to values that were no longer different from those after saline.

7.3.5 Long-term effects on regional hemodynamics

Pre-infusion values of MAP (mm Hg) and HR (bpm) for the different experimental groups just before the start of infusion on day 0 are summarized in table 7.4.

Table 7.4: Pre-infusion values (mean ± SEM) of MAP (mm Hg) and HR (bpm) of the different experimental groups in the long-term regional hemodynamic study.

	n	MAP	HR
0.9% NaCl	13	155±3	315±11
CGP 18 137A (1 mg/kg.d)	8	168±5	320±8
CGP 22 979A (10 mg/kg.d)	10	162±4	333±10

There were no significant differences between starting values in the different groups. Changes in MAP and HR together with flow and resistance changes in the three vascular beds during long-term treatment with saline, CGP 18 137A (1 mg/kg.d) and CGP 22 979A (10 mg/kg.d) are presented in fig. 7.5. The effects are expressed as percentage change from the values measured just before the start of the infusion.

During long-term infusion, both drugs significantly reduced MAP ($p<0.001$) compared to the saline-treated group. CGP 18 137A caused a rapid fall in blood pressure which was maximal 2-5 days after the start of the infusion. CGP 22 979A reduced MAP gradually during the whole infusion period. On day 5, the MAP reduction was almost the same for CGP 22 979A and CGP 18 137A (-21±2 and -26±4%, respectively). In

contrast to the effect of CGP 22 979A on HR, HR was significantly increased during long-term infusion of CGP 18 137A ($p<0.001$) compared to control animals. This increase was almost equal from the second day (+17±3%) to the end of the infusion (+18±4%).

During long-term CGP 18 137A treatment, flows in all vascular beds measured showed a trend to increase. Only in the case of the hindquarter, it reached the level of significance ($p<0.05$). After 3 days infusion of CGP 18 137A, HQF and MF returned to control values. A slight temporary flow increase in the kidney but not in the mesentery

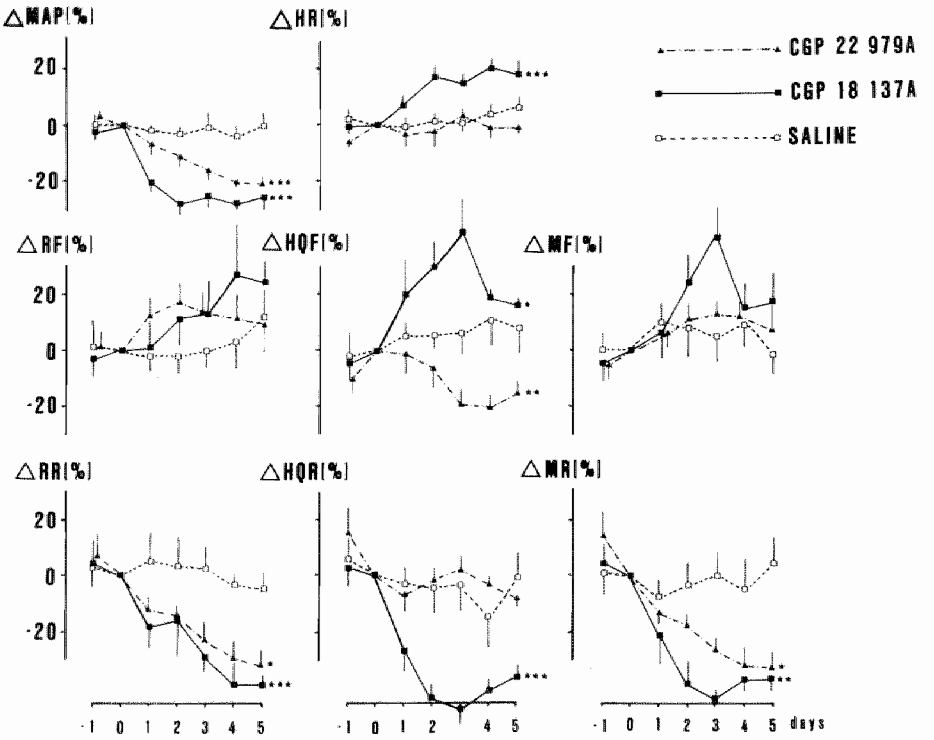


*Fig. 7.5: Effect of long-term infusion of CGP 22 979A (10 mg/kg.d), CGP 18 137A (1 mg/kg.d) and saline on mean arterial pressure (MAP), heart rate (HR), renal flow (RF), hindquarter flow (HQF), mesenteric flow (MF), renal resistance (RR, hindquarter resistance (HQR) and mesenteric resistance (MR) in conscious SHR. Significances are given in comparison with the saline infusion: *p<0.05; **p<0.01; ***p<0.001.*

*Table 7.5: Blood pressure (MAP, mm HG), heart rate (HR, bpm) and plasma renin concentration (PRC, ng AI/ml.hr) in the different experimental groups just before (day 0) and after a 4-day period of the respective drugs. Data are expressed as mean ± SEM. Significances are given in comparison to pre-infusion values: ***p<0.001.*

	n	Day	MAP	HR	PRC
0.9% NaCl	6	0	167±7	325±7	7.1 ±0.8
(0.1 ml)		4	168±7	338±9	7.5 ±1.1
CGP 18 137A	8	0	179±5	338±10	10.1±1.3
(1 mg/kg.d)		4	121±3***	396±19***	22.1±3.4***
CGP 22 979A	6	0	176±7	331±10	7.6 ±0.4
(10 mg/kg.d)		4	129±8***	332±14	8.7 ±1.6

was observed during infusion of CGP 22 979A. This in contrast to a significant reduction in HQF ($p<0.01$) during the long-term treatment of CGP 22 979A.

CGP 18 137A reduced RR, HQR and MR. In the hindquarter and mesenteric vascular bed, this reduction was maximal 2-3 days after the start of the infusion. In the case of CGP 22 979A, a reduction in RR and MR but no change in HQR was observed. In the kidney, this reduction was continuous and almost similar for both drugs during the whole infusion period. In the case of CGP 18 137A, the reduction in MR was stronger during the first 3 days of infusion. Thereafter, MR increased to the same level as in the case of CGP 22 979A.

7.3.6 Long-term effect on plasma renin concentration

The values of MAP, HR and PRC just before (day 0) and after 4 days infusion of the different experimental drugs are summarized in table 7.5. The data show no significant differences between the pre-infusion values of MAP and HR in the different experimental groups. There were no significant differences between the pre-infusion values of different treated groups. Long-term infusion of CGP 18 137A significantly decreased MAP about 32%, increased HR about 17% and increased

PRC by about 120% (compared to pre-infusion and control values). Long-term infusion of CGP 22 979A significantly decreased MAP ($p<0.001$) about 27%, but did not change HR and PRC.

7.4 Discussion

CGP 18 137A is a hydralazine-like vasodilator, whereas CGP 22 979A is the N-acetyl-L-gamma-glutamyl substituted analogue of CGP 18 137A (Hofbauer et al, 1985). Introduction of an N-acetyl-L-gamma-glutamyl moiety has been suggested as a prodrug approach for obtaining selectively high renal concentrations of drugs because the release of the active compound through hydrolysis by acylase and gamma-glutamyl transpeptidase occurs at a higher rate in the kidney than in other tissues (Orlowski et al, 1980). In a previous study (Hofbauer et al, 1985), CGP 22 979A was shown to lack vasodilatory properties in an in-vitro preparation in which CGP 18 137A exerted a dose-dependent vasodilation. Moreover, in that study it was shown that in the intact, anesthetized rat, CGP 22 979A caused a preferential renal vasodilation (Hofbauer et al;, 1985). Because cardiovascular reflexes which are grossly attenutated in anesthetized animals (Cox and Bagshaw, 1974; Zimpfer et al, 1981) may modify the responses, we studied systemic and regional hemodynamic effects of the possible selective renal vasodilator in conscious unrestrained SHR.

The acute central hemodynamic effects of CGP 18 137A and CGP 22 979A fit into two categories. CGP 18 137A and the highest dose (30 mg/kg) of CGP 22 979A cause an immediate reduction of MAP which is associated with an immediate fall in TPR. This resembles the response pattern which we observed after the administration of the classical arteriolar vasodilators hydralazine, dihydralazine and endralazine in conscious SHR (chapter 4). It also confirms the observations with regard to the acute non-selective vasodilatation after CGP 18 137A and high doses of CGP 22 979A in the regional hemodynamic study. In the case of CGP 18 137A, this pattern is in fact to be expected because it is a hydralazine-like compound. It seems likely that after 30 mg/kg CGP 22 979A the release of the parent compound from the prodrug occurs

at such a high rate that there is considerable spill-over of the active vasodilator into the general circulation.

Lower doses (<30 mg/kg) of CGP 22 979A do not cause an immediate reduction of MAP and TPR. They cause a late reduction of MAP with a tendency towards reduction of CO. This effect resembles that of diuretics in SHR (Struyker Boudier et al, 1983) which cause a primary decrease of SVI and CO. The resemblance fits with our observations with regard to renal function changes.

The diuretic-like effects of CGP 22 979A on central hemodynamics are not only observed after the low doses. In fact, the rise in CO after 30 mg/kg of the prodrug is much smaller than after CGP 18 137A. In a previous chapter, we have shown that the rise of CO after hydralazine depends upon activation of the sino-aortic baroreceptor reflex. Therefore, one explanation for the smaller rise in CO might be that CGP 22 979A interferes with the sino-aortic reflex. Alternatively, the effect on CO represents a summation of baroreflexes and the diuretic effect resulting in a lesser effect on CO.

Hydralazine (see chapter 4) and CGP 18 137A were fully comparable with regard to their blood pressure lowering potencies. Their regional hemodynamic effects, however, differed. Qualitatively both drugs behave as general vasodilators.Hydralazine induced larger decreases in RR and HQR than CGP 18 137A. This may imply that CGP 18 137A dilates other vascular beds to a larger extent than hydralazine. Alternatively, hydralazine may cause a larger drop in peripheral resistance at a comparable degree of blood pressure reduction. This can only be the case if hydralazine causes a larger degree of reflex rise in cardiac output. This does not seem likely because the tachycardia seen during a CGP 18 137A induced reduction of MAP tended to be greater than that observed during an equal reduction of MAP by hydralazine and 1.0 mg/kg CGP 18 137A increased CI more than 0.3 mg/kg hydralazine (see chapter 4).

CGP 22 979A was much less potent as an acute blood pressure lowering agent. Only doses of 10 to 30 mg/kg were capable of reducing MAP significantly. Only at the highest dose of 30 mg/kg, regional hemodynamic changes resembled those of CGP 18 137A. In doses of 1 to 10 mg/kg CGP 22 979A induced renal vasodilation without reducing MR and

HQR. After 30 mg/kg, however, all resistances decreased. Probably, this resulted from excessive release of CGP 18 137A from the kidney spilling over into the circulation.

Renal vasodilators have a potential importance for clinical use in disease states such as hypertension and renal failure. In the latter case, an increased renal resistance prevents normal glomerular filtration and the advantage of renal vasodilation may be a decrease in renal resistance without a concomitant fall in blood pressure that would further deteriorate renal function (Ackermann et al, 1982). Therefore, we studied the consequences of preferential renal vasodilation on renal hemodynamics and water and sodium excretion.

After bolus injections, CGP 22 979A increased ERPF dose-dependently, whereas CGP 18 137A caused a slight reduction. These observations confirm our findings in the regional hemodynamic study where we used 20 MHz pulsed Doppler probes to measure changes in renal blood flow after both compounds. The reduction of ERPF after CGP 18 137A was associated with a fall in GFR, whereas this parameter did not change within the first 1.5 h period after CGP 22 979A. In a previous study in anesthetized rats, Hofbauer et al (1985) noted a transient increase in GFR during the first 30 min after 10 mg/kg of CGP 22 979A. The reason for the discrepancy between results from the two studies may derive from the anesthetic used in one study, or from methodological differences. With regard to the effect of anesthesia, a dissociation between effects in conscious and anesthetized animals with regard to renal function has been reported for intrarenally administered acetylcholine and papaverine (Blaine, 1978; Blaine and Dunlay, 1981) although in the latter studies, GFR was affected similarly in conscious and anesthetized sheep. Alternatively, the differences may result from the different methods used, as the technique we used for the quantitation of GFR results in an integrated GFR value over a 1.5 hr period (Smits et al, 1982). Thus, transient changes will not be detected. However, ERPF also represents an integrated value over the same period. Therefore, it may be concluded that there is a dissociation between effects on GFR and plasma flow. This suggests that CGP 22 979A causes both afferent and efferent arteriolar dilatation in the kidney. Possibly, also the glomerular capillary filtration coefficient may be

reduced. On this basis, one would expect that the reduction in blood pressure that occurs after 30 mg/kg of CGP 22 979A would result in a decrease of GFR. However, our results suggest that the concomitant increase in renal blood flow compensates for the fall in glomerular capillary pressure because GFR in rats depends both upon arterial pressure (filtration pressure) and blood flow through the glomerulus (Marchand and Mohrman, 1980). After CGP 18 137A ERPF does not increase and, in fact, a fall in GFR is observed which confirms this hypothesis.

At 3 to 4.5 hr after CGP 22 979A, the flow increases did no longer persist. At this time, however, MAP had fallen in the groups injected with 10 and 30 mg/kg. Again, probably as a resultant of these two phenomena, GFR was reduced.

In spite of the unchanged GFR, a strong increase in water and sodium excretion was noted during the first 2 hr after CGP 22 979A. The increase in V and $U_{Na}V$ confirms earlier observations in conscious normotensive rats (Hofbauer et al, 1985). Although data on GFR and renal excretory function in the present study were obtained in separate groups of animals and thus do not allow calculation of FE_{Na}, the combined observations suggest a 2- to 4-fold increase in FE_{Na} after CGP 22 979A. Such an increase in FE_{Na} without changes in GFR as observed in the present study has been reported before and during renal vasodilatation by intrarenal infusions of substances like acetylcholine (Early and Friedler, 1965; Willis et al, 1968; Marchand et al, 1977; Blaine and Dunlay, 1981), papaverine (Blaine, 1978; Blaine and Dunlay, 1981) and several prostaglandins (Strandhoy et al, 1974; Oliver et al, 1979). Several mechanisms have been put forward to explain the decreased fractional sodium reabsorption. These include a washout of the juxtamedullary sodium gradient (Early and Friedler, 1965), an increase in peritubular capillary pressure (Strandhoy et al, 1974) and an increased renal interstitial pressure (Marchand et al, 1977). Our study was not designed in a way to discriminate between these possible mechanisms.

The present study shows that in conscious SHR, CGP 22 979A is a preferential renal vasodilator. Computer simulation studies of selective renal vasodilators suggest a more pronounced reduction in blood

pressure during long-term treatment induced by a slow reduction in TPR without a change in plasma angiotensin II levels (Struyker Boudier, 1980). The long-term regional hemodynamic studies showed that in the case of CGP 22 979A the acute preferential renal vasodilation developed into a long-term selective renal and mesenteric vasodilation. A long-term treatment of CGP 18 137A did not change the acute non-selective regional profile. It is unlikely that vasodilation in the mesentery during long-term CGP 22 979A treatment was induced by a spill-over of CGP 18 137A from the kidney into the circulation because such a spill-over would lead to a general vasodilation. We did not observe a vasodilation in the hindquarter vascular bed as was the case during long-term CGP 18 137A treatment.

Orlowsky et al (1980) observed an accumulation and metabolism of N-acyl-γ-glutamyl derivatives (CGP 22 979A like substances) preferentially in the kidney, but also in other organs e.g. pancreas, small intestine, liver and spleen. So, a possible explanation for the mesenteric vasodilation could be that long-term CGP 22 979A treatment leads to a preferential renal accumulation of the active compound CGP 18 137A directly followed by accumulation of CGP 18 137A in the splanchnic vascular bed formed by the same but slower mechanism as in the kidney but faster than in other tissue. Because no mesenteric vasodilation was seen after bolus injections of CGP 22 979A, this accumulation process has to be independent of plasma CGP 22 979A concentration. Measurements of plasma and tissue concentrations of CGP 22 979A and CGP 18 137A will be necessary to confirm this hypothesis.

In the treatment of hypertension, renal vasodilation may trigger a sequence of events that ultimately results in a decreased total peripheral resistance as a reflection of renal vasodilation and autoregulation of other vascular beds. Autoregulation in this context means that the individual tissues have an intrinsic ability to regulate their perfusion through changes in vascular resistance. Another possible explanation for the mesenteric vasodilation during long-term CGP 22 979A treatment could be autoregulation because no flow changes were observed in that vascular bed. This phenomenon was not seen in the hindquarters in spite of a significant flow decrease during a 5-day infusion of CGP 22 979A. In literature, autoregulation of almost

all vascular beds has been described extensively (reviews Schalekamp et al, 1985; Coleman et al, 1979). Few studies, however, are available describing differences in capability between vascular beds to autoregulate in one individual. Autoregulation was investigated in several organs in one preparation by Liard (1981). He increased cardiac output in conscious dogs by salt-loading. These animals exhibited on the first day of infusion a 25% increase of arterial pressure and cardiac output. Blood flows to the kidney and the splanchnic area were not significantly changed whereas skeletal muscle blood flow almost doubled. After several days, cardiac output returned towards control values but blood pressure remained elevated. Skeletal muscle blood flow as most other regional flows did not differ significantly from control values. These data indicate that the renal and mesenteric vascular beds immediately autoregulate by flow changes whereas the skeletal muscle vascular bed needs several days to autoregulate flow to control values. Our long-term observations correlate these previous findings.

Preliminary studies indicated that long-term administration of CGP 22 979A in SHR indeed leads to a chronic reduction in blood pressure (Smits et al, 1984). The present long-term regional hemodynamic studies confirm these observations. A slow continuous reduction in blood pressure was observed during long-term CGP 22 979A infusion with the advantage of no change in heart rate. This in contrast to the rapid sustained fall in blood pressure and tachycardia during CGP 18 137A administration.The long duration of tachycardia points to a lack of adaptation of the baroreflex to the prevailing levels of blood pressure. It has been shown in chapter 4 that the tachycardia after hydralazine is mediated through sino-aortic baroreceptors in SHR. Moreover, it was shown in chapter 4 that the activity of the baroreflex was only of very short duration, viz. 1-2 hr, whereas the fall in blood pressure may last up to 6-8 hr after a single dose of hydralazine. An explanation for these observations could be that hydralazine and hydralazine-like substances directly stimulate baroreflex mechanisms. In the case of a bolus injection, the plasma drug level after 1-3 hr was possibly too low for a plasma concentration dependent direct effect on baroreceptor reflex, whereas during chronic infusion,

plasma levels were sufficiently high enough. Plasma measurements will be necessary to confirm this hypothesis.

Another difference of CGP 22 979A treatment over the hydralazine-like vasodilator CGP 18 137A was its effect on plasma renin concentration. In contrast to the increase in PRC during CGP 18 137A treatment, no change was observed in the case of CGP 22 979A. The increase of renin during treatment with non-selective vasodilators is thought to result primarily from reduced renal perfusion pressure and a baroreflex mediated increase of sympathetic nervous activity (Gross, 1977). A baroreflex activation was not observed during the long-term CGP 22 979A treatment. Furthermore, it is unlikely that renal perfusion pressure decreased during the first period of CGP 22 979A infusion because systemic blood pressure decreased only to a small extent. With regard to the baroreflex and renal perfusion pressure, it is not surprising that PRC did not increase in the case of CGP 22 979A. Plasma renin release, however, also increases as a consequence of sodium depletion (Tarazi et al, 1970; Vaughan et al, 1973; Vaughan et al, 1978). This sodium depletion limits the long-term use of diuretic agents in the treatment of hypertension. In spite of the increase in sodium and water excretion during CGP 22 979A treatment, plasma renin activity did not increase. Possibly, tubular contents and transmural pressure in renal arterioles were in such a balance that renin release was not influenced in the kidney.

In conclusion, CGP 22 979A is a prodrug for the hydralazine-like vasodilator CGP 18 137A. Low doses (up to 10 mg/kg) have no immediate effect on blood pressure, whereas 30 mg/kg causes a fall in blood pressure as does CGP 18 137A. Contrary to the active compound CGP 18 137A, the renal vasodilator prodrug CGP 22 979A causes acute rises in renal blood flow and water and sodium excretion in spite of an unchanged GFR. The nature of the central hemodynamic changes for the active compound and the prodrug are different in as much that the active compound CGP 18 137A immediately reduces TPR and increases CO, whereas the prodrug gradually decreases peripheral resistance and causes less increase in CO, possibly due to the concomitant diuretic effect. In contrast to the long-term CGP 18 137A treatment, no tachycardia and plasma renin activity increase were observed in the case of

CGP 22 979A. The acute preferential renal vasodilation changed to a renal and mesenteric vasodilation. These results suggest that selective renal vasodilators may offer an important advantage to hydralazine-like vasodilators in the long-term treatment of hypertension.

CHAPTER 8

CONCLUDING REMARKS

In this thesis, the central and regional hemodynamic actions of several antihypertensive drugs were investigated in conscious unrestrained SHR. For the central hemodynamic measurements, rats were chronically instrumented with electromagnetic flowprobes and for the regional hemodynamics, miniaturized Doppler flowprobes were implanted. In the different chapters of this thesis the usefulness of these models to study hemodynamics was shown. The major advantage of these animal models is that they allow the continuous characterization of hemodynamic effects of antihypertensive drugs in undisturbed unanesthetized hypertensive small animals.

The acute central hemodynamic studies showed that the vasodilators hydralazine, dihydralazine, endralazine, CGP 18 137A and the calcium entry blockers verapamil, nifedipine and PY 108-068 decrease total peripheral resistance and induce a baroreflex mediated increase in cardiac output. This in contrast to the effects of both beta-adrenoceptor blockers propranolol and tertatolol. The latter substances decrease cardiac output and cause a baroreflex mediated increase in total peripheral resistance. In the case of beta-blockade the baroreflex mechanism can compensate the reduced cardiac output by a vasoconstriction. In the case of the vasodilators, the baroreflex mechanism is not able to completely compensate the reduced vascular resistance by the increase in cardiac output. Furthermore, the regional hemodynamic studies show that the baroreflex also plays an important role in the regional specificity of some vasodilators.

In these regional hemodynamic studies, flow changes were measured through the kidney, mesentery and skeletal muscle of the hind-

quarters of the rat. These three vascular beds receive about 60% of the total cardiac output.

The regional hemodynamic studies showed that hydralazine and the hydralazine-like substance CGP 18 137A cause a general vasodilation in all three vascular beds measured. This was not observed for the calcium entry blockers. These substances reduce hindquarter vascular resistance but do not affect renal and mesenteric resistance. In the central hemodynamic studies following CEBs, the reduction in total peripheral resistance was greater than the fall in hindquarter resistance. So, it is likely that the calcium entry blockers nifedipine, verapamil and PY 108-068 also dilate other vascular beds not included in our measurements.

In sino-aortic baroreceptor denervated animals the regional dilatory pattern was almost similar to that in intact animals after hydralazine. The calcium entry blockers showed after denervation at a 10-fold lower dose, a general vasodilatation in all three vascular beds. These results suggest that the baroreflex mechanism plays an important role in the short-term regional specificity of calcium entry blockers but not in that of hydralazine and CGP 18 137A.

Baroreceptor reflex activation by unilateral common carotid occlusion increases resistance in renal, hindquarter and mesenteric vascular bed. These results indicate that the regional specificity of calcium entry blockers cannot be explained by a different degree in sympathetic innervation of the vascular beds. Alternatively, a selective blockade of the sympathetic influence on skeletal muscle and possibly other, not measured, vascular beds may play a role.

A selective reduction of the sympathetic influence on vascular beds may also underly the regional specificity of the non-selective beta-blockers propranolol and tertatolol used in this thesis. Propranolol causes a comparable early increase in vascular resistance in the renal, hindquarter and mesenteric bed. Tertatolol, however, causes an early increase only in hindquarter resistance. In the central hemodynamic studies, a more pronounced early increase in total peripheral resistance was observed in the case of tertatolol as compared to propranolol suggesting involvement of other vascular beds.

After removal of the sino-aortic baroreceptor reflex control,

both beta-blockers behave similarly. A reduction in renal, an increase in hindquarter and no effect on mesenteric resistance was observed at a similar blood pressure reduction. These studies suggest that the regional specificity of tertatolol can be explained by a selective blockade of the sympathetic influence on the renal and mesenteric vascular beds but not on the hindquarter. Furthermore, these studies indicate that tertatolol increases a baroreflex mediated vasoconstriction in several not measured vascular beds, or that propranolol decreases or blocks a baroreflex mediated vasoconstriction in the other beds.

It may be assumed that the influence of a baroreflex-mediated interference with the hemodynamic effects of an antihypertensive drug disappears gradually, since it is known that the baroreceptor reflex resets to the prevailing blood pressure after a certain amount of time. So, during long-term treatment, the regional hemodynamic effects of the different antihypertensive drugs should be similar to those measured in sino-aortic baroreceptor denervated animals if no other reflexes are involved. For the vasodilators used in the studies described in this thesis, the regional hemodynamic effects in sino-aortic denervated animals were almost similar to the ones measured during long-term treatment. However, by comparing the acute and long-term regional hemodynamic studies of both beta-blockers propranolol and tertatolol, it seems that there is a difference in baroreceptor resetting in time. The long-term regional pattern of propranolol is almost comparable with that measured in intact animals after a bolus injection. However, the long-term regional pattern of tertatolol is almost similar as the pattern measured in the sino- aortic baroreceptor denervated animals. These observations suggest a more rapid resetting in the case of tertatolol and hardly any resetting in the case of propranolol.

Computer simulation studies hypothetized that renal vasodilation may trigger a sequence of events that ultimately results in a decreased total peripheral resistance as a reflection of renal vasodilation and autoregulation of other vascular beds. This hypothesis is based on the natriuretic and diuretic effects of renal vasodilation.

Our renal excretory function studies presented in chapter 7 confirm a diuretic and natriuretic effect for the preferential renal vasodilator CGP 22 979A. Because glomerular filtration rate did not change after CGP 22 979A we may assume that the natriuretic effect of this substance is a consequence of an increased tubular pressure.

The computer simulation study also suggests that selective renal vasodilation may have several great advantages above duretics in the treatment of hypertension. A longer therapy with diuretics leads, amongst others, to an increase in plasma renin activity but in the case of a renal vasodilation such a side-effect may not occur. Our long-term plasma renin concentration studies showed indeed that CGP 22 979A does not change plasma renin concentration. This is in contrast to the effect of its parent compound that increased plasma renin concentration.

Different mechanisms were suggested for the mesenteric vasodilation seen together with the renal vasodilation during long-term CGP 22 979A treatment. One possibility is that the mesenteric vasodilation is a consequence of autoregulation. However, another possible explanation for the mesenteric vasodilation could be that long-term CGP 22 979A treatment leads to a preferential renal accumulation of the active compound directly followed by accumulation of CGP 18 137A in the mesenteric vascular bed formed by the same but slower mechanism as in the kidney but faster than in other tissue. Measurements of plasma and tissue concentrations of CGP 22 979A and CGP 18 137A will be necessary to exclude one of the possibilities.

The studies presented in this thesis show that hydralazine and hydralazine-like substances and calcium entry blockers are non-selective vasodilators with several side-effects. Some of these unwanted effects can be explained by their non-selective action, e.g. headache, flushes, and obstipation. These observations indicate that an antihypertensive drug with a more selective site of action may offer great advantage in the treatment of hypertension. The data presented in this thesis on the selective renal vasodilator CGP 22 979A support this assumption. For this antihypertensive drug, a lack of side-effects such as fluid retention, tachycardia and increase in plasma renin concentration, was observed. Furthermore, these data support the

hypothesis underlying the computer simulation of the long-term antihypertensive properties of a selective renal vasodilation presented in chapter 1. These simulated effects of a preferential renal vasodilation can be explained by reversing the hypothesis of Borst and Borst-de Geus (1963) and Guyton (1974) for the development of hypertension. So far, the observed effects of the renal prodrug CGP 22 979A are in agreement with the simulation study. However, further investigations of the acute and long-term effects of the renal prodrug on blood volume and extracellular fluid volume may lead to more evidence for the simulated hypothesis.

SUMMARY

Hypertension is one of the riskfactors for cardiovascular diseases. In the established phase of essential hypertension the major hemodynamic change is an increase in total peripheral resistance. Several hypotheses which could explain this increase in total peripheral resistance are discussed in chapter 1.

Nowadays many antihypertensive drugs with various mechanisms of action are available. The usefulness of these antihypertensive drugs is often limited as a consequence of several side-effects occurring during the use of these drugs in therapy. Some of these side-effects such as a reflex-mediated increase in sympathetic activity counteract the hemodynamic effects of vasodilators.

It has been suggested that the relative lack of side-effects of some antihypertensive drugs is related to differences in hemodynamic profile of action. Chapter 1 hypothesizes that a vasodilator with a preferential renal site of action may be an antihypertensive drug with a minimum of side-effects. A selective renal vasodilator may reverse a series of events thought to be responsible for the development of hypertension in the hypothesis of Borst and Borst-de Geus (1963) and Guyton (1974).

In the studies described in this thesis we have paid attention to the possible differences of several antihypertensive drugs with regard to their central and regional hemodynamic effects. Furthermore, in some cases also renal hemodynamics and excretory functions were studied.

Chapter 2 describes the surgery and the methods used in the studies of this thesis. For the central hemodynamic measurements rats

were chronically instrumented with electromagnetic flowprobes and for the regional hemodynamic measurements Doppler flowprobes were implanted. In the regional hemodynamic studies flow changes were measured through the kidney, the mesentery, and the hindquarter of the rat. In all studies measurements were performed in undisturbed unanesthetized rats.

The baroreflex is an important mechanism in the short-term control of the cardiovascular system. Thus it may play an important role in the acute hemodynamic effects of antihypertensive drugs. Therefore, the effect of baroreceptor unloading on regional hemodynamics in conscious WKY rats was investigated as described in chapter 3. The baroreceptors were unloaded in unilaterally denervated rats by contralateral common carotid occlusion, thereby activating the baroreceptor reflex. The results indicate that baroreceptor unloading leads to a vasoconstriction in all vascular beds studied.

Chapter 4 describes the hemodynamic effects of hydralazine and some hydralazine-like arteriolar vasodilators dihydralazine and endralazine. These agents lead to an immediate fall in blood pressure caused by a fall in total peripheral resistance. This is associated with a baroreflex mediated increase in cardiac output and heart rate. Endralazine is slightly more active than hydralazine. The regional hemodynamic studies indicate that the fall in total peripheral resistance after hydralazine is caused by a generalized reduction of resistance in the renal, hindquarter and mesenteric vascular bed. Furthermore, these studies indicate that the general vasodilation seen after hydralazine is the result of an additive effect of a strong direct vasorelaxation by hydralazine and a vasoconstriction by baroreflex activation.

In chapter 5 hemodynamic effects of the calcium entry blockers verapamil, nifedipine and PY 108-068 are described. All three agents cause a dose-dependent fall in blood pressure and total peripheral resistance. The fall in blood pressure triggers a baroreflex mediated rise in heart rate and cardiac output which is probably counteracted by direct cardiac effects in the case of verapamil. In intact animals the acute fall in total peripheral resistance is related primarily to a decrease in vascular resistance of the muscular bed. However, the

calcium entry blockers cannot be regarded as selective dilators of this vascular bed since in baroreflex denervated SHR and during long-term treatment in intact SHR the degree of vasodilation was similar in all three vascular beds studied. No effect on plasma renin concentration was observed during chronic verapamil treatment.

Chapter 6 describes the hemodynamic effects of the beta adrenoceptor blockers propranolol and tertatolol in conscious SHR. The central hemodynamic studies show that after tertatolol administration the cardiac output decreases immediately whereas the change in MAP consists of an early rise followed by a later decrease. These data confirm previous reports from our laboratory on the time dependent effects of the beta adrenoceptor blocker propranolol. The early rise in MAP, however, was more pronounced in the case of tertatolol as compared to propranolol. Previous studies have shown that the lack of acute decrease in blood pressure is caused by a baroreflex mediated rise in total peripheral resistance. The regional hemodynamic studies show that propranolol causes similar increases in vascular resistances in the renal, hindquarter and mesenteric beds. Tertatolol, in contrast, causes an early increase only in hindquarter resistance. After removal of the sino-aortic baroreflex control, propranolol and tertatolol induce similar effects, i.e. a reduction in renal, an increase in hindquarter and no effect on mesenteric resistance. These results indicate that tertatolol reduces the baroreflex mediated constriction in the renal and mesenteric vascular beds in intact animals. For tertatolol the long-term regional pattern in intact animals is almost similar to the acute regional hemodynamic effects measured in denervated animals. This is in contrast to propranolol of which the long-term hemodynamic effects are comparable with the acute regional hemodynamic effects in intact animals. These results suggest a more rapid baroreceptor resetting during long-term treatment with tertatolol as compared to propranolol.

Tertatolol thus protects the kidney from a hypoperfusion as was observed for propranolol. This difference in renal perfusion could lead to differences in renal excretory function. Both beta-blockers, however, induce an acute diuresis and have no effect on glomerular filtration rate. These results suggest a beta-adrenoceptor mediated

reduction in tubular reabsorption. During long-term treatment a reduction in plasma renin concentration was observed in the case of propranolol. Tertatolol, in contrast, did not affect plasma renin concentration.

In chapter 7 the hemodynamic effects of the renal vasodilator prodrug CGP 22 979A and its parent compound CGP 18 137A are described. Sequential hydrolysis by acylase and glutamyl-transpeptidase of CGP 22 979A is needed in order to generate the hydralazine like vasodilator CGP 18 137A. These reactions occur at a higher rate in the kidney than in other tissues (Orlowski et al, 1980), possibly resulting in a selective accumulation of the parent compound in the kidney.

Low doses of CGP 22 979A (up to 10 mg/kg) have no immediate effect on blood pressure, whereas 30 mg/kg causes a maximal fall in blood pressure comparable to the fall induced by CGP 18 137A (1 mg/kg). Contrary to the parent compound CGP 18 137A, the renal vasodilator prodrug CGP 22 979A causes acute rises in renal blood flow and water and sodium excretion in spite of an unchanged GFR. The nature of the central hemodynamic changes for the active compound and the prodrug is different inasmuch as the active compound immediately reduces total peripheral resistance and increases cardiac output, whereas the prodrug gradually decreases peripheral resistance and causes a smaller increase in cardiac output, possibly due to the concomitant diuretic effects. In contrast to the effects observed during long-term CGP 18 137A treatment, no tachycardia and no increase in plasma renin concentration are observed in the case of CGP 22 979A. The acute preferential renal vasodilation changes to renal and mesenteric vasodilation during long-term CGP 22 979A treatment.

In chapter 8 the implications of the different regional hemodynamic profiles of antihypertensive drugs are discussed in relation to the long-term treatment of hypertensive disease.

The lack of the side-effects induced by vasodilators, i.e. fluid retention, tachycardia and increase in plasma renin concentration, may offer great advantage for a selective renal vasodilator in the treatment of hypertension.

SAMENVATTING

Hoge bloeddruk is een risikofaktor voor hart- en vaatziekten. De belangrijkste hemodynamische verandering in de gestabiliseerde fase van essentiële hypertensie is de toegenomen totale perifere weerstand. Verschillende hypothesen die deze toename in totale perifere weerstand zouden kunnen verklaren, zijn beschreven in hoofdstuk 1.

Tegenwoordig is een groot aantal bloeddrukverlagende middelen met een verschillend werkingsmechanisme beschikbaar. Door diverse bijwerkingen is de bruikbaarheid van deze antihypertensiva vaak beperkt. Sommige van deze bijwerkingen, zoals de reflex gemedieerde toename in sympathische zenuwaktiviteit en de renale retentie van zout- en water, werken het hemodynamische effekt van antihypertensiva tegen.

Er wordt wel gesuggereerd dat het uitblijven van ongewenste effekten van sommige antihypertensiva samen hangt met een verschillend hemodynamisch werkingsprofiel. Hoofdstuk 1 bespreekt onder andere de hypothese dat een vaatverwijder met een bij voorkeur renaal aangrijpingspunt mogelijk minimale bijwerkingen heeft. Een selektieve renale vaatverwijder keert mogelijk de volgorde van de gebeurtenissen die verantwoordelijk zijn voor het ontstaan van hoge bloeddruk volgens de hypothese van Borst en Borst-de Geus (1963) en Guyton (1974) om.

In de studies beschreven in dit proefschrift werd aandacht besteed aan mogelijke verschillen van diverse antihypertensiva met betrekking tot hun centrale en regionale hemodynamische effekten. Verder werd in enkele gevallen ook de renale hemodynamika en zout- en wateruitscheiding bestudeerd.

Hoofdstuk 2 beschrijft de chirurgie en methoden die gevolgd werden in de studies van dit proefschrift. Voor de centrale hemodyna-

mische metingen werden ratten chronisch geinstrumenteerd met elektromagnetische flowprobes en voor de regionale hemodynamische metingen met Doppler flowprobes. In de regionale hemodynamische studies werden flowveranderingen gemeten door de nier, het splanchnische vaatbed en het achterlijf van de rat. In alle studies werden de metingen verricht in wakkere, vrij bewegende ratten.

De baroreceptorreflex is een belangrijk mechanisme voor de kontrole op korte termijn van het kardiovaskulaire systeem. Dientengevolge zou deze reflex mogelijk een belangrijke rol kunnen spelen in de akute hemodynamische effekten van antihypertensiva. Daarom werd het effekt van aktivatie van de baroreceptorreflex op de regionale hemodynamika in wakkere Wistar-Kyoto ratten bestudeerd, zoals beschreven in hoofdstuk 3. In unilateraal gedenerveerde ratten werd door een kontralaterale okklusie van de arteria carotis de baroreflex geaktiveerd. De resultaten laten zien dat een ontlading van baroreceptoren tot een vasokonstriktie in alle bestudeerde vaatbedden leidt.

Hoofdstuk 4 beschrijft de hemodynamische effekten van hydralazine en enkele hydralazine-achtige arteriolaire vaatverwijders, dihydralazine en endralazine. Deze stoffen veroorzaken een onmiddellijke bloeddrukdaling door een afname van de totale perifere weerstand. Dit gaat samen met een baroreflex gemedieerde toename van het hartminuutvolume en hartfrekwentie. Endralazine is wat aktiever dan hydralazine. De regionale hemodynamische studies laten zien dat de afname van de totale perifere weerstand na hydralazinetoediening bestaat uit een daling van de vaatweerstand in de nier, het splanchnische gebied en het achterlijf van de rat. Verder laten deze studies zien dat de algehele vaatverwijding na hydralazinetoediening het resultaat is van een sterke, direkt door hydralazine veroorzaakte vaatverwijding en een baroreflex gemedieerde vaatvernauwing.

In hoofdstuk 5 zijn de hemodynamische effekten van de calciuminfluxremmers verapamil, nifedipine en PY 108-068 beschreven. De drie substanties veroorzaken een dosisafhankelijke daling van de bloeddruk en totale perifere weerstand. De bloeddrukdaling leidt tot een baroreceptorreflex gemedieerde toename in hartfrekwentie en hartminuutvolume. Deze toename wordt in het geval van verapamil mogelijk tegengewerkt door een direkt effekt van deze stof op het hart. In intakte

dieren is de akute daling van de totale perifere weerstand direkt gerelateerd aan een daling van de weerstand in het spiervaatbed. In de baroreceptor gedenerveerde spontaan hypertensieve ratten en tijdens de chronische toediening van calciuminfluexremmers wordt echter een vergelijkbare vaatverwijding waargenomen in alle drie de bestudeerde vaatbedden. De calciuminfluxremmers kunnen dus niet als selektieve vaatverwijders voor het spiervaatbed beschouwd worden. Gedurende chronische verapamilbehandeling is geen effekt op de plasma renine-koncentratie waargenomen.

Hoofdstuk 6 beschrijft de hemodynamische effekten van de beta-blokkers propranolol en tertatolol in wakkere spontaan hypertensieve ratten. De centrale hemodynamische studies laten zien dat na tertato-loltoediening het hartminuutvolume onmiddellijk daalt, terwijl de bloedrukverandering bestaat uit een akute toename gevolgd door een daling. Deze gegevens komen overeen met de resultaten van eerder door ons laboratorium gepubliceerde studies met betrekking tot de tijds-afhankelijke effekten van de betablokker propranolol. De akute bloed-druktoename is echter meer uitgesproken in het geval van tertatolol ten opzichte van propranolol. Eerder gepubliceeerde studies hebben laten zien dat het uitblijven van een akute daling van de bloeddruk het gevolg is van een baroreflex gemedieerde toename in totale peri-fere weerstand. De regionale hemodynamische studies laten zien dat propranolol een vergelijkbare toename in vaatweerstand veroorzaakt in de nier, het splanchnische gebied en achterlijf van de rat. Tertatolol daarentegen veroorzaakt slechts een toename van de vaatweerstand in het achterlijf. Na het wegnemen van de baroreceptorreflex veroorzaken tertatolol en propranolol vergelijkbare effekten, namelijk een vermin-dering in renale vaatweerstand, geen effekt in splanchnische vaatweer-stand en een verhoging van de weerstand in het achterlijf. Deze resul-taten leiden tot de konklusie dat tertatolol de baroreflex gemedieerde vasokonstriktie afzwakt in de nier en het splanchnische gebied van intakte dieren. Voor tertatolol is het regionale patroon in intakte dieren op lange termijn vrijwel vergelijkbaar met de akute regionale hemodynamische effekten gemeten in gedenerveerde dieren. Dit in tegen-stelling tot propranolol, waarvan de lange termijn hemodynamische effekten beter vergelijkbaar zijn met de akute regionale hemodyna-

mische effekten in intakte dieren. Deze resultaten suggereren voor tertatolol een snellere baroreceptor resetting gedurende lange termijn behandeling dan voor propranolol.

Tertatolol beschermt dus de nier voor een verminderde perfusie, zoals die was waargenomen voor propranolol. Een dergelijk verschil in renale perfusie zou tot verschillen in renale water- en zoutuitscheiding kunnen leiden. Dit is echter niet gevonden. Beide betablokkers veroorzaken namelijk een akute diurese en hebben geen effekt op de glomerulaire filtratiesnelheid. Deze resultaten suggereren een beta-adrenoceptor gemedieerde vermindering in tubulaire reabsorptie. Gedurende de lange termijn behandeling met propranolol werd een daling van de plasma reninekoncentratie waargenomen. Tertatolol daarentegen heeft geen invloed op de plasma reninekoncentratie.

In hoofdstuk 7 zijn de hemodynamische effekten van de renale prodrug CGP 22 979A en zijn aktieve verbinding CGP 18 137A beschreven. Gedeeltelijke hydrolyse van CGP 22 979A door acylase en glutamyltranspeptidase is nodig om de hydralazine-achtige vaatverwijder CGP 18 137A te genereren. Deze reakties verlopen sneller in de nier dan in ander weefsel (Orlowski et al, 1980) wat mogelijk een akkumulatie van de actieve verbinding in de nier tot gevolg heeft. Lage doseringen CGP 22 979A (tot 10 mg/kg) hebben geen onmiddellijk effekt op de bloeddruk, terwijl 30 mg/kg een daling van de bloeddruk veroorzaakt vergelijkbaar met de daling geinduceerd door CGP 18 137A (1 mg/kg). In tegenstelling tot de aktieve verbinding CGP 18 137A veroorzaakt de renale produrg CGP 22 979A een akute toename van de renale doorbloeding en water- en zoutuitscheiding bij een onveranderde glomerulaire filtratiesnelheid.

De centrale hemodynamische veranderingen voor de aktieve verbinding en de prodrug zijn in zoverre verschillend dat de aktieve verbinding onmiddellijk de totale perifere weerstand doet dalen en het hartminuutvolume doet stijgen, terwijl de prodrug langzaam de totale perifere weerstand doet dalen en een kleine toename in hartminuutvolume veroorzaakt, mogelijk als gevolg van het hierboven beschreven diuretisch effekt. In tegenstelling tot de effekten waargenomen gedurende de lange termijn CGP 18 137A behandeling is in het geval van CGP 22 979A geen tachykardie en geen toename in plasma renine-aktiviteit waargenomen. Terwijl in de akute experimenten de vaatverwijding prefe-

rentieel renaal is, wordt bij de chronische behandeling met CGP 22 979A een renale en splanchnische vaatverwijding gezien.

In hoofdstuk 8 zijn de implikaties van de verschillende regionale profielen van de antihypertensiva beschreven in relatie tot de lange termijn behandeling van hoge bloeddruk.

Doordat bij selektieve renale vaatverwijding bijwerkingen, zoals vochtretentie, tachykardie en een toename in plasma reninekoncentratie, uitblijven, biedt deze groep van vaatverwijders mogelijk grote voordelen bij de behandeling van hoge bloeddruk.

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CURRICULUM VITAE

Hubert N.M.W. Nievelstein werd op 16 juli 1954 te Kerkrade geboren.
Zijn HBS-B opleiding volgde hij aan het Antonius Doctor College te Kerkrade, waar in 1972 het diploma behaald werd.
In hetzelfde jaar ging hij wiskunde studeren aan de Katholieke Universiteit te Nijmegen.
In 1974 veranderde hij van studierichting en begon hij aan dezelfde universiteit zijn studie scheikunde.
Het kandidaatsexamen (S_2) werd afgelegd in 1978.
In 1981 legde hij het examen MO-B scheikunde met goed gevolg af.
Vervolgens werd het doctoraal examen scheikunde met als hoofdvakken biochemie (onder leiding van prof.dr. H. Hoenders en dr. J. Bindels) en farmacochemie (onder leiding van prof.dr. J. van Rossum; practische stage bij Organon BV te Oss onder leiding van drs. A. Coert en dr. J. van der Vies) afgelegd in juni 1983.
Vanaf juni 1983 tot maart 1987 is hij als wetenschappelijk assistent in dienst van de Nederlandse Organisatie voor Zuiver Wetenschappelijk Onderzoek verbonden geweest aan de vakgroep farmacologie (hoofd: prof.dr. H.A.J. Struyker Boudier) van de Rijksuniversiteit Limburg te Maastricht.
Sinds 1 maart 1987 is hij in dienst van Duphar BV te Weesp en werkzaam op de afdeling farmacologie.

LIST OF PUBLICATIONS

Full papers

1. HAJ Struyker Boudier, HNMW Nievelstein, H van Essen, JFM Smits. Calcium antagonists: systemic and regional hemodynamic effects in conscious spontaneously hypertensive rats (SHR). J Hypertension 2 (suppl 3), 527-529, 1984.

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4. HNMW Nievelstein, H van Essen, CM Tyssen, JFM Smits, HAJ Struyker Boudier. Systemic and regional hemodynamic actions of calcium entry blockers in conscious spontaneously hypertensive rats. Eur J Pharmacol 113, 187-198, 1985.

5. HMNW Nievelstein, H van Essen, R Hornsveld, HAJ Struyker Boudier, JFM Smits. Effects of the renal vasodilator prodrug CGP 22 979A and its parent compound CGP 18 137A on renal and central hemodynamics in conscious, spontaneously hypertensive rats. J Pharmacol Exp Ther 235, 778-782, 1985.

6. HNMW Nievelstein, CM Tyssen, JFM Smits, HAJ Struyker Boudier. Regional hemodynamic effects of the beta-adrenoceptor blockers tertatolol and propranolol in conscious, spontaneously hypertensive rats. Arch Int Pharmacodyn 282, 118-129, 1986.

7. HAJ Struyker Boudier, H van Essen, HNMW Nievelstein, JFM Smits. The role of baroreflex activations in the regional hemodynamic effects of the beta blockers tertatolol and propranolol in conscious, spontaneously hypertensive rats. Am J Nephrol 6 (suppl 2), 25-29, 1986.

8. HAJ Struyker Boudier, JCS Kleinjans, LML le Noble, HMN Nievelstein, JFM Smits. Rate-controlled cardiovascular drug administration and action. Rate-controlled drug administration and action. (ed: HAJ Struyker Boudier). CRC Press, Boca Raton (Fl), USA, 1986, pp 171-203.

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11. H Nievelstein, C Tyssen, J Smits, H Struyker Boudier. Long-term regional hemodynamic effects of the beta-blockers tertatolol and propranolol in conscious, spontaneously hypertensive rats (In preparation).

12 H Nievelstein, C Tyssen, J Smits, H Struyker Boudier. Long-term regional hemodynamic effects of the renal vasodilator prodrug CGP 22 979A and its parent compound CGP 18 137A in conscious, spontaneously hypertensive rats (In preparation).

Abstracts

1. H Nievelstein, A Coert, J van der Vies, MA Blankenstein, J Bolt-de Vries. Effects of hyperprolactinemia on the accessory sexual organs of the male rat. Proc Fed Verg Med Wet Ver 1983.

2. HNMW Nievelstein, JFM Smits, HAJ Struyker Boudier. Effects of nifedipine and hydralazine on regional blood flows in conscious, spontaneously hypertensive rats (SHR). Pharmaceut Wbl 5, 264, 1983.

3. H Nievelstein, C Tyssen, H Struyker Boudier, J Smits. Differential baroreflex modulation of regional responses to vasodilator drugs in conscious, spontaneously hypertensive rats (SHR). Pharmaceut Wbl 6, 226, 1984.

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